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Determination of long-term effects of consecutive effective soil solarization with vesicular arbuscular mycorrhizal (VAM) on white rot disease (*Sclerotium cepivorum* Berk.) and yield of onion

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ABSTRACT

The aim of this study was to increase the yield in the fields solarized two years earlier (2007) by reducing the white rot disease in onion resulted from *Sclerotium cepivorum* caused by Vesicular Arbuscular Mycorrhizal (VAM), *Glomus intraradices* fungus. Study was conducted in two different experiments (the first experiment where tomato was grown after solarization; the second experiment where lettuce and pepper in previous growing seasons were grown after solarization). Pearl bulb onions were grown with four repetitions according to split-split plot design (2008). In the experiments, the consecutive effects of solarization were considered as main and of sub-VAM and *S. cepivorum* as mini plots. With the artificial inoculation of *S. cepivorum*, sufficient disease symptoms were not detected in the onion bulb; however, it reduced the thickness of leaves and stems. And for these two development parameters, results were found significant ($P<0.01$). Bulb onion yield decreased by 11% in the first experiment and 16% in the second experiment with the *S. cepivorum* inoculation. The effect of solarization on yield in the second year was higher than in the third year. In the second consecutive season (experiment 1), both of shallot and bulb onion yield increased by 25%, and in the third season (experiment 2), yield increased by 18% for bulb onion. Leaf and stem thickness increased with VAM, but decreased by *S. cepivorum*. Results were found to be significant ($P<0.01$) for both the experiments. Consequently, thanks to VAM, product yield increased in both the experiments, and the increments were determined as 22% for shallot; as 25% for bulb onion yield in the second experiment. The effects of solarization combined with VAM increased bulb onion yield by 50%, compared to without both of them. Among the combinations, the highest yield was achieved with the Sol-VAM-non-*S. cepivorum* application in comparison to non-solarized-non-VAM-*S. cepivorum*, through this application provided an increase of 54.41% for the first and 91.13% for the second experiment. In conclusion, the effect of solarization, gradually became less effective, in the second and third production seasons. Development of onion alternating in these seasons and loss of yield caused by *S. cepivorum* could be reduced with artificial VAM inoculation. However, this effect must have depended on the relation between plant species and mycorrhizal colonization in earlier seasons.

Key words : Onion, *Sclerotium cepivorum*, soil solarization, Vesicular Arbuscular Mycorrhizal (*Glomus intraradices*)

INTRODUCTION

Intensive onion production, the main problem is the yield reduction caused by soil borne pathogens and weeds. This problem could be eliminated by soil fumigation with chemical and/or crop rotation. However, it was known that chemicals had harmful effect on ecology. The most common chemical used in fumigation was methyl-bromide, but was recommended to be forbidden by 2005 because it damages the stratospheric ozone layer (Katan, 1999). Soil solarization is a term that refers to disinfestations of soil by the heat generated from trapped solar energy (Katan, 1987). This method eradicates or reduces soil-borne pathogens and weed seed germination by thermal inactivation, and the purpose of the

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hot season of the soil can be covered under transparent polyethylene sheets (Lalitha *et al.*, 2003). Soil solarization has a great potential for increasing onion yield decreasing soil-borne disease severity, which is white rot caused *Sclerotium cepivorum*, in the Mediterranean region (Satour *et al.*, 1989).

However, the destruction of beneficial organisms such as vesicular arbuscular mycorrhizal (VAM) fungus may also occur, thereby reducing positive effects of solarization (Schreiner *et al.*, 2001). The symbiotic relationship between VAM and the roots of higher plants contributes significantly to plant nutrition and growth (Smith and Read, 2008). Onion is a high cash value crop with a very shallow root system that is frequently irrigated and fertilized with high N rates (Halvorson *et al.*, 2008). These shallow root tips depend on mycorrhizal fungi with hyphal P uptake for bulb onion production in the poor soil nutrients (Sharma and Adholeya, 2000). In addition, some studies have indicated that AM fungi have several beneficial functions on the water relations of onions (Al-Karaki *et al.*, 2006) and which suggest great potential of AM fungi for mitigating water stress of onions in drought regions (Bolandnazar *et al.*, 2007). Several studies have shown that the association of root systems of crop plants, including onion, with AM-fungi can increase the ability of plants to absorb water and nutrients and can result in increased biomass production and yields using artificial soil inoculated with mycorrhiza (Afek *et al.*, 1991) which have been destroyed or reduced by fumigation or solarization (Schreiner *et al.*, 2001).

Ecosystem in this region is brought by the relationship that exists between mycorrhizal and the plant; VAM protects the plant from diseases caused by soil-borne pathogens (Harrier and Watson, 2004). In a previous study, it was reported that there had been a significant negative correlation between Allium white rot disease incidence and vesicular-arbuscular mycorrhizal (AM) *Glomus intraradices* root colonization in organic soils (Jaime *et al.*, 2008).

The aim of this study was to determine the long-term effects of consecutive effective soil solarization with vesicular arbuscular mycorrhizal (VAM) on white rot disease (*Sclerotium cepivorum* Berk.) and yield of onion in solarized area where lettuce, pepper and tomato were grown in previous years.

MATERIALS AND METHODS

Experimental Area

The study was conducted as two experiments in 2009 in the fields of Agricultural Faculty of Dicle University in Diyarbakýr/Turkey, which were solarized two years earlier (2007). Lettuce (2007) and pepper (2008) were grown earlier after solarization on the fields of the first experiment. On the other hand, only tomato was grown earlier (2008) after solarization on the fields of the second experiment.

Plant Material, Vesicular-Arbuscular Mycorrhizal (VAM) and Pathogen

Pearl (little bulb) onion (*Allium cepa* L. cv. Lice) was used as plant material in both the experiments. *Sclerotium cepivorum* (Berk.) was obtained from Plant Protection Research Institute, Bornova/IZMIR; Vesicular-Arbuscular Mycorrhizal (VAM), *Glomus intraradices* Schenk and Smith were obtained from Soil Department of Agricultural Faculty of Çukurova University.

Experimental Pattern

Both the experiments were conducted in a split-split-plot design with four replications. The experiment was established in solarized and non-solarized soil as main plots; VAM infested and non-VAM as sub-plots, which were inoculated with or without *S. cepivorum* as mini parcel. The same plots infested with VAM from the previous years were also infected with the same fungus (*G. intraradices*) used as VAM plots.

Plantation of Pearl Onions with VAM Inoculation

On 12 March 2009, the pearl onions were transplanted in the field in depth to 10 cm with 25 x 10 cm rows spacing and each plot included 80 plants. Firstly 10 g of the combination with VAM (*Glomus intraradices*) (andesitic tuff, soil and compost in 6 : 3 : 1 proportion) was put into holes, pearl onions were placed with their stems down and then the holes were filled with soil. Onion plots with or without mycorrhiza were irrigated just after the transplanting.

Preparation of Inoculum from S. *cepivorum* and Inoculation

On 24 February 2009, at first, the isolate of *S. cepivorum* was proliferated on potato-dextrose agar (PDA) in petri dishes at 22±2°C, and then saturated into wheat grain culture in Erlenmeyer containing autoclaved wheat (1000 g cracked wheat+800 ml water) afterwards the grains were inoculated with *S. cepivorum* isolates and incubated at 22±2°C for incubation. And then afterwards, development of sclerotia was observed during mycelial growth of *S. cepivorum*.

On 25 March 2009, *S. cepivorum* fungus grown in wheat grain culture was pieced and inoculated to experimental plots. Inoculum saturated with wheat grain of 190 g/m^2 density was put into the 5-10 cm holes between plant rows and mixed with soil. Surface watering was made on plots just after the inoculation.

Determination of the Disease Incidence

Infected onion bulbs during harvesting were selected, and disease incidence was rated on 0-5 scale where 0=healthy, 1=bulb covered with mycelium but not rotted, 2=1-25% of the bulb rotted, 3=25-50% of the bulb rotted, 4=50-75% of the bulb rotted and 5=75-100% of the bulb rotted (Tian and Bertolini, 1995). Disease severity scores were converted into percentage as follows :

$$\text{Disease severity (\%)} = \frac{\text{Total points score} \times 100}{\text{Total number of bulbs} \times \text{Highest score}}$$

Observation of Plant Growth and Determination of Yield

Plant height (cm) : Measured by rules from the bottom of white part of white green onion above the surface of soil.

Leaf thickness (mm) : Measured by digital cumpas in three young leaf not the oldest.

Stem diameter (mm) : Middle of the white part of shallot measured by digital cumpas.

Plant weight (g) : Each shallot (green leaves) weighted one by one after isolated the root soil.

Shallot yield (kg/ha) : Each plot harvested as green onion and weighted by digital balance.

Bulb onion yield (kg/ha) : Each plot harvested and dried 10 days in the field than weighted by digital balance.

RESULTS AND DISCUSSION

Observation of White Rot Disease Caused by *S. cepivorum*

Disease observation was carried out on onion bulbs after harvest (13 July) 115 days after artificial inoculation (31 March) and the results are given in Tables 1 and 2. In Table 2, disease incidence was seen to be higher in plots inoculated with *S. cepivorum* than not inoculated ones in both the two experiments ($P<0.01$). Disease incidence of onion bulbs in the inoculated fields was found to be lower than expected. This may have been caused due to the fact that the soil temperature increased rapidly 45 days after inoculation. As a consequence, disease incidence decreased because infection time didn't overlap sufficiently with onion growing period. In a previous study, it was reported that *S. cepivorum* germinated best below 20°C and in high humidity (Kim *et al.*, 2009). Also, the pathogen was reported to have become inactive above 25°C (Davis and Aegerter, 2009). Disease incidence could reach to 80% in different phenologic periods, depending on climate and inoculum potential (Ponce-Herrera *et al.*, 2008).

Effects of S. *cepivorum* on Onion Growth and Yield

Leaf and stem thickness was reduced by the artificial inoculation of *S. cepivorum*. And, for these two development parameters, results were found significant ($P<0.01$) in the second experiment.

Same structure was effective in product yield, as well. Bulb onion yield was decreased

Table 1. Effects of solarization, VAM and *Sclerotium cepivorum* on the development of onion (2009)

Treatment	1st experiment[a]			2nd experiment[b]		
	Plant length (cm)	Leaf thickness (mm)	Stem thickness (mm)	Plant length (cm)	Leaf thickness (mm)	Stem thickness
Solarized	51.16A	3.78A	13.22A	55.25	3.30	14.89
Non-solarized	46.22B	3.26B	11.70B	56.00	3.86	15.73
Solarization	*	*	**	NS	NS	NS
VAM	49.11	3.73	12.75	57.67A	3.88A	16.30A
Non-VAM	48.27	3.32	12.18	53.58B	3.29B	14.32B
VAM	NS	NS	NS	**	**	**
Sol. VAM	52.92	4.23A	13.93A	56.63	3.48	15.36B
Sol. non-VAM	49.41	3.32B	12.50AB	53.87	3.12	14.43B
Non-Sol. VAM	45.31	3.22B	11.56B	58.71	4.27	17.25A
Non-Sol. non-VAM	47.13	3.31B	11.85B	53.28	3.46	14.21B
S x VAM	NS	*	**	NS	NS	*
Inoculation of *S. cepivorum*	48.96	3.50	12.64	55.32	3.25B	14.61B
Non-inoculation of *S. cepivorum*	48.42	3.54	12.28	55.93	3.91A	16.01A
S. cepivorum	NS	NS	NS	NS	**	**
Sol. *S. cepivorum*	52.70A	3.57	13.53	56.25A	3.10	14.38
Sol. non-*S. cepivorum*	49.63AB	3.98	12.91	54.26A	3.51	15.41
Non-Sol. *S. cepivorum*	45.23B	3.43	11.75	54.40A	3.41	14.85
Non-Sol. non-*S. cepivorum*	47.21B	3.10	11.66	57.60B	4.32	16.61
S x Sc	**	NS	NS	*	NS	NS
VAM x Sc	NS	NS	NS	NS	NS	NS
S x VAM x Sc	NS	NS	NS	NS	NS	NS

[a]Effect of solarization in the second production season.
[b]Effect of solarization in the third production season.
*Significant at P=0.05 and P=0.01 level, respectively. NS : Not Significant.

Table 2. Effects of solarization, VAM and *Sclerotium cepivorum* on white rot disease and yield in onion (2009)

Treatment	1st experiment[a]			2nd experiment[b]		
	Disease incidence (%)	Shallot yield (kg/ha)	Bulb onion yield (kg/ha)	Disease incidence (%)	Shallot yield (kg/ha)	Bulb onion yield (kg/ha)
Solarized	0.89	17881.2A	31369.2A	1.43B	23450.0	36006.2A
Non-solarized	0.89	14218.6B	24971.5B	0.70A	25275.0	30612.5B
Solarization	NS	**	**	**	NS	*
VAM	0.40A	16681.2	29187.4	0.38A	26750.0A	37068.7A
Non-VAM	1.38B	15418.6	27153.3	1.75B	21975.0B	29550.0B
VAM	**	NS	NS	**	**	**
Sol. VAM	0.63	19475.0A	34625.0A	0.26A	24625.0B	38825.0
Sol. Non-VAM	1.15	16282.4B	28113.6A	2.60B	22275.0B	33187.5
Non-Sol. VAM	0.17	13887.4B	23750.0B	0.50A	28875.0A	35312.5
Non-Sol. Non-VAM	1.61	14550.0B	26193.1AB	0.91A	21675.0B	25912.5
S x VAM	NS	*	*	**	*	NS
Inoculation of *S. cepivorum*	1.61B	16618.6	26545.4B	2.00B	23137.4B	30475.0B
Non-inoculation of *S. cepivorum*	0.17A	15481.2	29795.4A	0.13A	25587.4A	36143.7A
S. cepivorum	**	NS	*	**	*	**
Sol. *S. cepivorum*	1.50	19525.0A	29954.5	2.59C	22925.0	33737.5
Sol. non-*S. cepivorum*	0.28	16237.4B	32784.0	0.26A	23975.0	38275.0
Non-Sol. *S. cepivorum*	1.73	13712.4C	23136.3	1.41B	23350.0	27212.5
Non-Sol. non-*S. cepivorum*	0.58	14725.0BC	26808.0	0.00A	27200.0	34012.5
S x Sc	NS	*	NS	NS	NS	NS
VAM-*S. cepivorum*	0.63A	17262.4	27056.8	0.76B	24520.0	34412.5
VAM-non-*S. cepivorum*	0.28A	16100.0	31318.1	0.00A	28975.0	39725.0
Non-VAM-*S. cepivorum*	2.60B	15975.0	26034.0	3.24C	21750.0	26537.5
Non-VAM-non-*S. cepivorum*	0.43A	14862.4	28272.7	0.26AB	22200.0	32562.5
VAM x Sc	**	NS	NS	**	NS	NS
S x VAM x Sc	NS	NS	NS	**	NS	NS

[a]Effect of solarization in the second production season.
[b]Effect of solarization in the third production season.
*Significant at P=0.05 and P=0.01 level, respectively. NS : Not Significant.

residual inoculum of VAM due to mutualistic symbiosis between the root system of tomato grown in previous year. In fact, tomato has a strong root system which can develop deeply (Esau, 1977); and plants of this anatomic type have little symbiotic and mycorrhiza requirements (Brundrett, 2009).

Determination of the Interaction between Solarization and VAM

In the first experiment, there were significant interactions between solarization and VAM for leaf ($P<0.05$) and stem thickness ($P<0.01$), shallot ($P<0.05$) and bulb onion yield ($P<0.05$), but insignificant for plant length (Tables 1 and 2). In the second experiment, these were significant for stem thickness ($P<0.01$) and shallot yield ($P<0.01$), but insignificant for other growth parameters (Tables 1 and 2).

In the first experiment, the highest bulb onion yield was obtained with solarization and VAM combination, and in spite of the lowest bulb onion yield with non-solarization-VAM combination. This increase was determined as 45.78%.

In the second experiment, the highest bulb onion yield was also obtained with solarization and VAM combination, and in spite of the lowest bulb onion yield with non-solarization and non-VAM combination. The highest yield obtained was 49.83% higher than the ones with the lowest yield. Increase in bulb onion yield per hectare obtained with artificial VAM inoculation applied after solarization was compatible with the previous studies. In fact, earlier studies suggested eradication or reduction of mycorrhizal population in soil by fumigation or solarization could be recovered by artificial inoculation of VAM, could induce a better root growth and result in an increment in bulb onion yield (Afek *et al.*, 1991).

Effects of Solarization, Vesicular-Arbuscular Mycorrhizal (VAM) and *S. cepivorum* on Onion Yield

In the first experiment, interactions among soil solarization, VAM inoculation and *S. cepivorum* infection on shallot and bulb onion yield are given in Fig. 1. The highest bulb onion yield per hectare was obtained with solarization-VAM-non-*S. cepivorum* combination. This application provided an increase of 54.42% in yield compared to non-Sol- non-VAM-*S. cepivorum* combination in which disease inoculation was applied without solarization and VAM. Also, solarization-VAM-non-*S. cepivorum* combination, which was the highest bulb onion yield, 68.37% higher than non-Sol-VAM-*S. cepivorum* combination in which the lowest bulb onion yield was obtained.

In the second experiment, a similar order was obtained (Fig. 2). In the experiment, the highest bulb onion yield was achieved with Sol-VAM-non-*S. cepivorum* combination, and the lowest bulb onion yield was obtained with non-Sol-non-VAM-*S. cepivorum* combination in

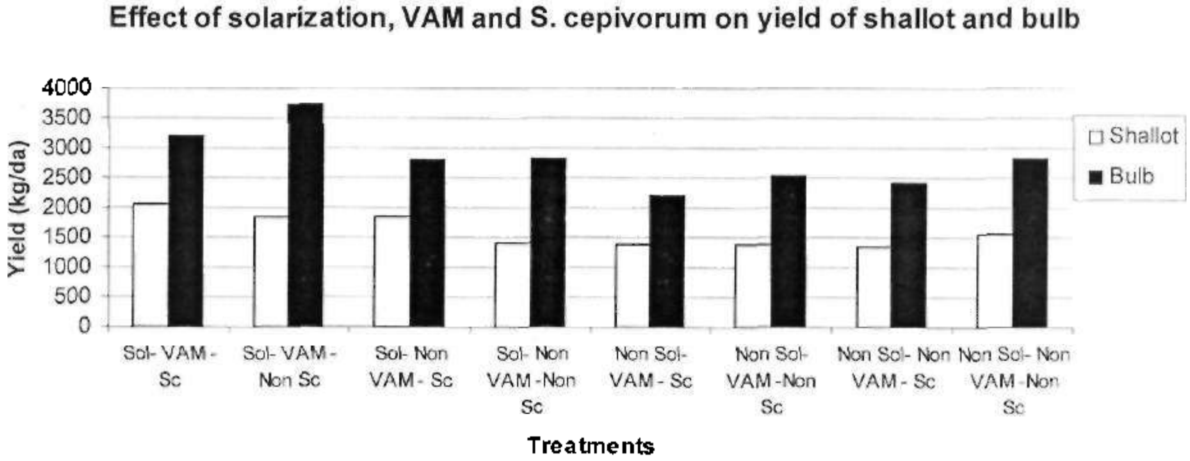

Fig. 1. Effects of solarization, VAM and *Sclerotium cepivorum* on product yield of green and dry onions (1st experiment).

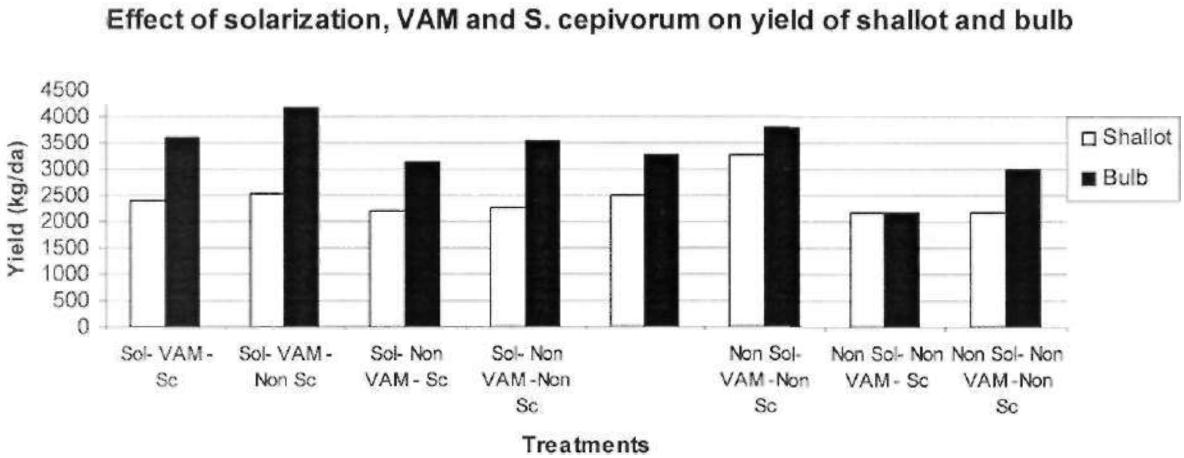

Fig. 2. Effects of solarization, VAM and *Sclerotium cepivorum* on product yield of green and dry onions (2nd experiment).

which *S. Cepivorum* inoculation was applied without solarization and VAM. The increase of yield rate in plot of the highest yield obtained was 91.13% higher than the ones with the lowest yield.

CONCLUSION

In conclusion, the effect of solarization, gradually became less effective, in the second and third growing seasons. Development of onion alternating in these seasons and loss of shallot and bulb onion yield caused by *S. cepivorum* could be reduced with artificial VAM inoculation. However, this effect must have depended on the relation between plant species and mycorrhizal colonization in earlier seasons.

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