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Response of Chive (*Allium schoenoprasum*) to AM Fungal Application Following Soil Solarization under Field Conditions

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Abstract

Four field experiments were conducted to examine the effect of soil solarization and chemical Dazomet on chive production following arbuscular mycorrhiza (AM) fungal application. The experiments were carried out in four different farm conditions and solarization treatment varied between four to eight weeks starting at different dates during the summer of 1999. Reduction in the indigenous AM population was evident in all sites following the solarization treatment and reduction in plant growth was observed at early stages of growth development up to the third harvest. Inoculation of chive with *Glomus intraradices* based inoculant reduced the incidents of growth retardation and resulted in plant growth suitable for export grade at the first and second harvests. A minimum of 2.5% (v/v) of inoculum is required to obtain this result. Mycorrhization significantly increased

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yield in four different chive cultivars in Dazomet treated and solarized soil. We suggest that the growth retardation effect induced by soil solarization and Dazomet pre-treatment to soil could be abolished by pre-colonizing chive plants with mycorrhiza before introducing into the field.

Keywords: Arbuscular mycorrhiza, chive, Dazomet, *Glomus intraradices*, soil solarization, soil fumigation

1. Introduction

Chive (*Allium schoenoprasum*) is cultivated as a fresh herb for the spice industry in Israel. During the growth season, plants are infected by the fungus *Pyrenochaeta terrestris*, causal agent of pink root disease resulting in a significant economic loss. To avoid any chemical residues in the harvested parts of the plant (leaves), conventional fungicide applications are forbidden and alternative technologies like soil solarization are favored. Commonly used fungicidal agents like, methyl bromide and metam sodium though effective, are highly detrimental to the environment with long-term toxicity of its breakdown products. Soil solarization was developed primarily for controlling soil borne pathogens and weeds (Katan, 1981; Katan and DeVay, 1991) and frequently results in an increase in plant growth response, which, is assumed to be due to pathogen control (Katan and DeVay, 1991). Soil solarization is the process of heating soil by capturing the sun's energy under clear plastic. The resulting increase in soil temperature can reduce the population of soilborne plant pathogens and weeds. The effects of solarization on soilborne plant pathogens occur at soil depth of up to 40–60 cm (Katan and DeVay, 1991). Little is known about the effect of soil solarization on beneficial organisms, including mycorrhizal fungi. It was demonstrated that after 4 weeks of solarization treatment, the AM fungal population declined significantly at soil depth of 0–40 cm (Bendavid-Val et al., 1997). In case of ectomycorrhiza fungi, this treatment eliminated from the forest nursery these symbiotic organisms from a depth of 15 cm of the soil profile (Soulas et al., 1997).

Bendavid-Val et al. (1997) found a significant decrease in AM fungal propagules and mycorrhizal colonization in onion and carrot roots growing in solarized soils. Root colonization was not evident until 6 weeks after transplanting the plants in treated soil. Moreover, in the above study, the reduction in AM fungal population was associated with plant growth retardation in onion and carrot roots which have a shallow root spread relative to deep root system crops like wheat. Pullman et al. (1981) found a decrease in AM fungus propagules after solarizing soil at one site, but not

another. This was attributed to the higher soil temperatures attained in solarized plots at the first site, as compared with the second. Other studies have shown either no effects or increased AM colonization in solarized soils that reached similar temperatures to those at which suppression of AM fungi was found (Afek et al., 1991; Nair et al., 1990).

In Israel and other hot climates, soil solarization is commonly used in onion fields to control diseases such as pink root caused by *Pyrenochaeta terrestris* and *Fusarium*, as well as, nematodes and other soil microbes (Katan et al., 1980; Rabinowitch et al., 1981; Satour et al., 1989). The present study was conducted to determine the effects of soil solarization and chemical disinfestations on the viability and infective potential of endogenous AM fungi. The effect of colonization, development and growth of chive in commercial agriculture practices due to application of soil solarization *per se* was studied. Responsiveness of five different chive cultivars was also studied for AM contribution to plant development when exposed to solarized and chemical treatment.

2. Materials and Methods

Field experimental details

The experiments were conducted in farmers' commercial greenhouse in the Yavneel area, Schinock (Farm S), Dudu (Farm D), Costizcky (Farm C) and in the experimental farm of the Hebrew University in Rehovot (Farm R). Soil characteristics of Yavneel were clay with 0.51 organic matter, 34% clay, 46% silt and 20% sand with pH of 8.2. In Farm R soil characteristic was sand with 0.12 organic matter, 4% clay, 2% silt, 94% sand with pH of 7.9.

Experimental design and soil treatment

Factorial-designed experiments were done to study the effects of soil solarization and mycorrhizal inoculation on chive development and production. Plots were arranged in a randomized block design with four replications per treatment, each of 2 adjacent beds (10 m long × 5 m wide). Solarization was achieved by mulching soil with clear polyethylene, 0.035 mm-thick sheets (Genegar, Israel). Each experiment was mulched for a period as indicated. Soil temperature data were continuously collected by micrologger (CR-21X, Campbell Scientific Inc., Logan, Utah). Dazomet (Basamid granular, BASF, Germany), at a rate of 45 gm/m^2 was spread on the soil surface with a manual driven spreader and then was incorporated by rototiling. After incorporation, the soil surface was mulched using clear plastic film 0.035 mm for 7 days.

Plant material and AM inoculum

Chive (*Allium schoenoprasum*) cv "Denfeld" was used as main crops in this study unless indicated otherwise. The AM fungus, *Glomus intraradices* [Schenck & Smith], was cultivated in the Volcani Center, Bet Dagan, Israel in association with Sorghum (*Sorghum bicolor* L.) as a host. The inoculum consisted of spores, hyphae and infected roots introduced to the growing media by mixing (v/v) with a commercial potting mix (peat moss: vermiculite at 7:3, v/v). Inoculum was incorporated at the rate of 10% (v/v) in the growing media or as stated otherwise and AM inoculation was carried out before sowing. Chive plants were grown in this potting mix at the nursery stage, for 4 weeks after germination and the resultant chive seedlings were then transplanted into the field according to the experimental design. At harvest time, leaves were cut 4 cm above the soil surface within an area of 0.9 m^2. Fresh weight was recorded and gradation for its quality was done subsequently. When root systems were evaluated, the entire root system of ten plants within the sample area were pooled, washed, cleared and then stained with trypan blue (Phillips and Hayman, 1970), examined for mycorrhizal colonization under a dissecting microscope and the percent colonization estimated using the gridline intersection method as described by Giovannetti and Mosse (1980).

Statistical analyses

Data was first analyzed by analysis of variance to test possible interaction among the main effects, (solarization, AM inoculation) followed by mean separation using t-test. All analyses were performed with the SAS program (SAS Institute Inc., Cary, NC, release 8,0 for personal computer) at P 0.05.

3. Results

AM survival and establishment in solarized soil

To determine the effect of solarization (timing and duration) on viability of endogenous AM population to infect chive plants, solarization treatments were initiated at different dates, started from 1st of June and extended for 4, 6 and 8 weeks (Farm D; Fig. 1). The daily soil temperature during the solarization treatment was recorded (at a depth of 10 cm) and maximal soil temperature during the month of June was 40°C whereas, during the month of August was 47°C (Fig. 2). AM colonization rates of non-inoculated plants were correlated with the solarization intensity: higher colonization levels were obtained in roots of plants grown in soil treated from the middle of June and extended for 4

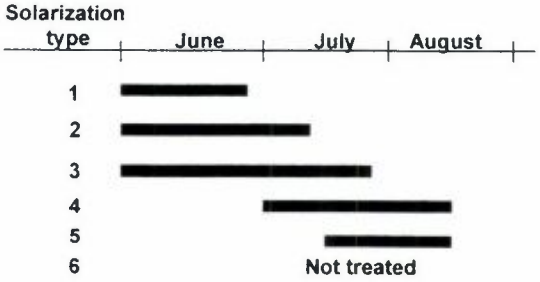

Figure 1. Scheme of timing and duration of soil solarization treatment in Farm D during the summer of 1999. Type 1, 2 and 3 were initiated on 1st June 1999, and was extended for 4, 6 and 8 weeks, respectively. Type 4 was initiated on 1st of July and extended for 6 weeks and Type 5 was initiated on 15th July and extended for 4 weeks. Type 6 was not treated by any solarization and served as a non-solarized control.

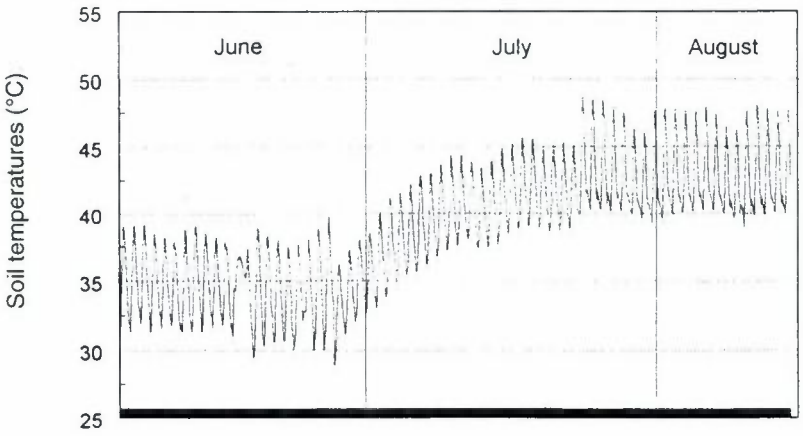

Figure 2. Daily soil temperature during the whole solarization process. Values were taken at a depth of 20 cm, under the polyethylene cover during the summer of 1999.

and 6 weeks (Type 4 and 5; Fig. 3) with no significant differences to the non-solarized plots (Type 6). Moreover, lower colonization was evident in roots of plants grown in plots solarized during the middle of summer for 5 weeks (Type 5; Fig. 3). These results indicate that a stronger solarization treatment at the middle of summer reduces the native AM population and thus does not support any subsequent infection of non-inoculated plants.

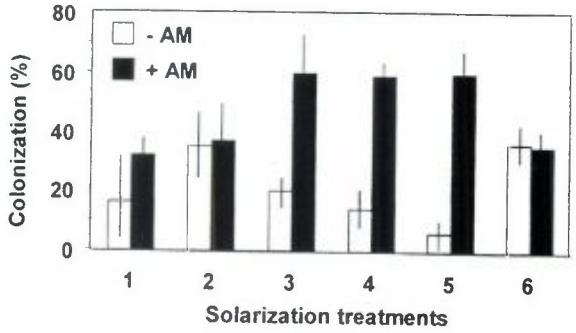

Figure 3. Colonization rate of AM fungi in chive roots grown on different solarization treatments. Plants were non-inoculated (–AM) or pre-inoculated (+AM) with mycorrhizal inoculant prior to transplantation and evaluation was carried 6 weeks after transplantation (Farm D). Values are mean of 5 replicates and vertical bars represent ±SE.

When chive plants pre-infected with *G. intraradices* were introduced in to different solarized plots, the effect of the treatment on root colonization was notably different (Fig. 3). A higher AM colonization level of the inoculated plants was found in plots of Type 3 to 5 treatment, whereas lower AM establishment capacity was evident in roots of plants grown in Type 1 and 2 treatments. The fact that the solarization treatment *per se* was effective, which can be inferred by the observation that the colonization in non-mycorrhizal plants was similar to the levels obtained in the pre-inoculated plants (Fig. 3). This clearly demonstrates the presence of an active suppressive factor(s) (biological/chemical) in the soil.

Effect of AM inoculation on plant development and growth in solarized soils

Growth of chive plants on solarized soil resulted in a significant reduction in plant development as compared to non-solarized soil. Statistically significant reduction in plant development due to solarization treatments which, was carried out during the month of August was observed (Table 1). Plants grew slower and a higher degree of necrotic regions on the leaves could be seen within two weeks from the date of transplantation. Solarization treatments that were initiated on the 1st of June for 4 or 6 weeks or July for 4 weeks did not affect the plant growth significantly. The effect of soil solarization was obvious as seen by the reduction in the yield of chive when no mycorrhization was done. This was rescued by the presence of AM inoculation in both the harvest (1 and 2) types (Table 1).

Table 1. Effect of soil solarization intensity and duration: solarization types on chive fresh weight in the presence (+AM) and absence (–AM) of mycorrhizal inoculant. Results were obtained from 1st and 2nd harvests in Farm D. Values are mean of 5 replicated plots and different letters within main effect denoted significant differences ($P<0.05$).

Solarization type	Harvest 1			Harvest 2		
	–AM (g/m^2)	+AM (g/m^2)	Average (g/m^2)	–AM (g/m^2)	+AM (g/m^2)	Average (g/m^2)
1	67	219	143 a	208	468	338 ab
2	75	243	159 a	233	505	369 a
3	90	237	164 a	192	402	297 b
4	37	173	105 b	170	430	300 b
5	24	218	121 b	175	430	300 b
6	98	222	160 a	265	483	374 b
Average	65 b	218 a		196 a	454 b	

Table 2. Effect of AM inoculation on chive fresh weight yield inoculated in the nursery (Farm S). Inoculum level in the growing media was 10% (v:v) and plants were transplanted to soil treated with soil solarization for 4 weeks (initiated at middle of June 1999). Numbers are means of 5 replicates. High and total refers to the exports quality and total yield, respectively.

	Inoculation	Harvest number (g/m^2)		Total yield (g/m^2)	Percent increase (%)
		1	3		
High	–AM	411 b	453 a	864 b	
	+AM	600 a	489 a	1089 a	26
Total	–AM	847 b	991 a	1838 b	
	+AM	1246 a	1012 a	2258 a	23

Different letter within each column at each grade denote significant differences among inoculation treatments ($P<0.05$).

In another independent experiment where solarization was initiated in the middle of July and was extended for 4 weeks, AM contribution was detected at two harvests, 1 and 3. The effect of AM at a rate of 10% on Farm S was

Table 3. Effect of inoculum level on chive fresh weight during three consecutive harvests. Plants were inoculated in the nursery by varying the inoculum level in the growing media. Plants were transplanted to 4 weeks treated solarized soil, the treatment of which was initiated during the middle of June, 1999. Numbers are means of 5 replicates and values present high (export) and total grades (including export) of the whole production.

	Inoculum level (%)	Harvest number (kg/m^2)			Total yield (kg/m^2)	Percent increase (%)
		1	2	3		
High	0	0.89 a	0.82 a	0.99 b	2.70 b	
	2.5	1.02 a	0.89 a	0.87 b	2.78 b	2
	5.0	1.03 a	0.94 a	1.05 b	3.02 b	11.6
	10.0	0.85 a	0.93 a	1.24 a	3.02 b	11.6
Total	0	1.82 b	1.66 b	2.41 b	5.89 b	
	2.5	2.10 ab	1.79 ab	2.29 b	6.18 b	4.9
	5.0	2.20 a	1.91 a	2.75 ab	6.86 a	16.5
	10.0	1.85 b	1.86 ab	3.07 a	6.78 a	15.1

Different letter within each column and each grade denote significant differences among inoculum level ($P<0.05$).

Table 4. Effect of AM inoculation on fresh weight of 4 different chive cultivars grown on solarized, non-solarized and Dazomet treated soil. Experiment was carried in Rehovot Farm (Farm R) and solarization was carried out during June–August of 1999. Chive seedlings were planted on Sept 1 and harvested 6 weeks later. Values are mean of five replicate plots and different capital and small letters denoted significant differences ($P<0.05$) between AM application treatments and soil treatments, respectively.

Cultivar		Soil treatments (g/m^2)		
		Non-solarized	Solarized	Dazomet
Spirling	–AM	62.1 Aa	49.8 Bb	37.1 Bb
	+AM	45.5 Ab	97.8 Aa	81.4 Aa
Grolau	–AM	53.1 Ba	50.1 Aa	26.1 Bb
	+AM	103.5 Aa	62.6 Ab	47.2 Ab
Hillau	–AM	65.2 Aab	77.7 Ba	58.8 Bb
	+AM	49.9 Ac	135.5 Aa	88.9 Ab
Villau	–AM	14.3 Ba	17.6 Ba	12.2 Ba
	+AM	24.5 Aa	25.9 Aa	22.4 Aa

evaluated and its effect on total or export yield grade. Following pre-inoculation, mycorrhizal plants yielded 26 and 23% higher biomass of export (high) grade and total biomass, respectively (Table 2). The fact that AM inoculation contributed to crop development at the first harvest, more than the third one (Table 2) suggests that the combined beneficial effect of the optimized solarization treatment and mycorrhization are more pronounced during the early stages, when crop establishment is of the foremost importance. Moreover, AM contribution was evident at both export grade and total yield, which was at a similar rate (about 23–26%) suggesting that mycorrhization did not benefit plant performance on a selective basis.

In order to quantify the lowest level of inoculation required in the growth media during seedling preparations (at the nursery) for maximal mycorrhizal beneficial response, a dilution experiment was carried out. Three levels of inoculum were applied, 2.5%, 5% and 10% (v/v) in the media mix and its effect on chive plants was tested on solarized soil in Farm C. Higher colonization was obtained at 10% inoculum application level relative to 2.5% although results were not statistically significant (data not shown). Moreover, along three consecutive harvests, a higher yield was obtained in all inoculated treatments relative to the control, which was significant at the 5 and 10% inoculation treatment (Table 3). Similar to the results obtained in Farm S, mycorrhizal colonization contributes to the export grade the same as to the total yield of these experimental plots.

Response of five different chive cultivars to AM application

In Farm R (Rehovot), the effect of mycorrhizal colonization on four different chive cultivars was tested on solarized or chemically treated (Dazomet) soil. After soil treatment and before planting, soil samples from 20 and 40 cm below ground from all treatments (solarized, Dazomet, and non-treated control) were sampled for the viability of the indigenous AM population. Soil samples were homogenized for each depth and filled in 0.5 l pots seeded with melon (*Cucumis melo*), which served as test indicator plants. After 5 weeks of growth, no intraradical AM colonization was observed in the melon roots of the test plants at both, solarized and Dazomet soils of all the depths. However, AM infection was observed in two third of the samples taken from the non-solarized soils (data not shown). It could be concluded that by both, Dazomet and solarization treatment the indigenous mycorrhizal population and growth retardation syndrome could be expected in this field. To evaluate the potential of different cultivars to benefit from mycorrhization after solarization and chemical disinfection, four different cultivars were planted in the treated soils. Each cultivar was tested with and without mycorrhiza and plant performance was

evaluated at the first harvest. When plants were treated with the fumigant Dazomet, yields were dramatically lower as compared to non-treated control (Table 4). In the Dazomet treatment, all cultivars tested responded significantly to the mycorrhizal application. These results indicate that the fumigant induces a growth retardation syndrome similar to solarization treatment. In solarized soil, mycorrhiza contributed significantly to all cultivars except, Grolau. In non-treated soil, mycorrhizal application did not affect significantly the cultivars Hillau and Spirling. It could be concluded that the chive cultivars respond differentially to the mycorrhizal response.

4. Discussion

This study shows that the growth retardation effect in chive, induced by soil solarization and chemical application can be abolished by mycorrhizal technology. Furthermore, soil solarization which affects viability and colonization potential of indigenous AM fungi, reduced productivity at early stages of growth thus reducing productivity up to 25% of the growth potential. Similar effects of chemical fungicides on AM fungi were noted by Menge (1982) and demonstrated in Israel (Dodd et al., 1983; Krikun et al., 1990; Bendavid-Val et al., 1997). Similarly, it was noted before that, reduction in plant growth following fungicide treatment was attributed to the destruction of the AM fungi (Schreiner et al., 2001). We confirmed that applications of Dazomet (substitute of methyl bromide in future agricultural application) also resulted in a complete loss of root colonization potential by indigenous AM fungi. Solarization reduced mycorrhizal colonization and at the same time was able to promote the introduced AM inoculant (Fig. 3).

A significant reduction in indigenous AM fungal infective propagules was found in control plots after 4 weeks of the soil solarization treatments (Bendavid-Val et al., 1997). This dramatic reduction in the number of ineffective propagules was found to be more pronounced in the solarization treatments carried out in the middle of summer (Type 5; Fig. 1). One of the possible explanations for this phenomenon may relate to the effect of the high light intensity and warm weather as was noted from the soil temperature data (Fig. 2) under the clear polyethylene. These conditions in the absence of a plant host, may affect mostly vegetative hyphae as was shown in our earlier work (Bendavid-Val et al., 1997). Any fungal propagules leading to root colonization had to survive the soil treatment. It is therefore suggested that solarization affects AM propagules vigor rather than eradicates the entire fungal population. The reported effects of sublethal heat stress on other soilborne fungi occurred over relatively short periods, of 1–3 days (Freeman and Katan, 1988; Lifshitz et al., 1983). Populations of the fungal pathogens like

Verticillium dahlia and *Phytophthora fragaria* could survive under these conditions especially over the winter if not eradicated over the same stage (Pinkerton et al., 2000).

Soil solarization affects plant performance at early stages of plant growth, which was correlated to plant adaptation following transplantation. The fact that a minimum amount of inoculum is required in order to enhance chive plants under field conditions can be explained by the fact that in each chive plug 20–30 seeds are introduced in the nursery and being considered as one seedling plug for the plantation. If high colonization rate is desirable, one should consider the multi-seedling situation and count for all the germinated seeds introduced to the plug, that requires a high inoculum titer. Application of high cfu permits in the current study fast AM establishments in the roots during the short period the seedling exist in the nursery. We suggest that our ability to demonstrate the beneficial effect for chive may be related to the high level of inoculation in the growing mix (200–230 propagules per gm inoculum). Evidently, chive seedlings after being introduced in the treated soil could benefit from mycorrhization immediately after transplanting as was evident from the different soil conditions (clay vs. sand), and by different cultivars of the chive variety (Table 4).

In conclusion, now when methyl bromide is going to be banned for agricultural practice, alternative strategies need to be considered for soil disinfection. This would include the use of soil solarization technology which reduces the viability of AM fungal propagules and as a result of it reduces the potential of plant development at an early stage of crop development. In order to significantly contribute to the chive production potential growers should consider the use of AM inoculant before transferring it to pretreated solarized or fungicidal treated plots, to overcome the growth retardation syndrome commonly encountered during such agricultural practice.

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