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Full Length Research Paper

Effects of solarization and vesicular arbuscular mycorrhizal fungus (VAM) on phytopthora blight (*Phytophthora capsici* leonian) and yield in pepper

Ismail Cimen[1], Vedat Pirinc[2]*, Abuzer Sagır[1], Cagdas Akpinar[3] and Sadettin Guzel[1]

[1]Dicle University, Agricultural Faculty, Department of Plant Protection, 21280, Diyarbakır, Turkey.
[2]Dicle University, Agricultural Faculty, Department of Horticulture, 21280, Diyarbakır, Turkey.
[3]Çukurova University, Agricultural Faculty, Department of Soil, 01330, Adana, Turkey.

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In this work, effects of soil solarization and vesicular arbuscular mycorrhizal (VAM) fungus, *Glomus intraradices* on diseases caused by *Phytophthora capsici* Leonian in pepper plants and crop yields were studied. At the end of the growth season, 82.5% mortality of plants and 47.7% of yield loss caused by *P. capsici* were obtained in control plots. At the beginning of the vegetative period, plant mortality caused by *P. capsici* in solarized plots was less than that in non-solarized plots, but it was higher at the end of the experiments. The total crop yield, however, increased to 20.9% by solarization. At the beginning of the growth season, anthocyanin production, early flowering and fruit settings were observed in the seedlings inoculated with VAM. Plant mortality caused by *P. capsici* was inhibited by 69.4% in plants inoculated with VAM fungus, but this rate decreased to 14.9% at the end of the experiment. On the other hand, total yield increased to 40.4% in plots infested with *P. capsici*, but treated with VAM. The total yield increased to 49.9% in pathogen free solarized + VAM inoculated grown plants plots in comparison to pathogen free non-solarized + non-VAM inoculated grown plants plots. This increase was 42.8% in solarized + VAM inoculated grown plants plots, which were infested with *P. capsici*. Total yield was 22.7% in solarized + VAM inoculated grown plants plots without *P. capsici* in comparison to non-solarized plots + non-VAM inoculated grown plants plots but infected with *P. capsici*. The yield loss caused by *P. capsici* in pepper was decreased by means of long-term effect of soil solarization with artificially VAM inoculation.

Key words: Soil solarization, vesicular arbuscular mycorrhizal, *Glomus intraradices*, *Phytophthora capsici,* yield, phytopthora blight, pepper.

INTRODUCTION

The main problem in pepper growing regions 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 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).

It is possible to use solar energy instead of fumigation, and it is also beneficiary using it. 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 (Tekin and Cimen, 2001; Lalitha et al., 2003).

By thermal inactivation, solarization is made available to decrease the crown rot disease caused by *Phytophthora capsici* L. (Yucel, 1995), which is the most dangerous disease affecting pepper cultivation in Turkey. However, the destruction of beneficial organisms such as

*Corresponding author. E-mail: vedpir@dicle.edu.tr.

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), and has been shown to increase the productivity of a variety of agronomic crops including pepper (Ortas et al., 2001). Root surface is increased by adding these fungi into soil (Thannuja et al., 2001).

In recent years, the practices of using artificial soil inoculated with mycorrhiza are very common. Following application of VAM, increase in yield and plant growth was reported in some cultural plants (Afek et al., 1991; Ortas et al., 2003). Ecosystem in this region is brought by the relationship that exists between mycoorhizal and the plant; VAM protects the plant from diseases caused by soil-borne pathogens (Harrier and Watson, 2004).

The aim of the present work is to increase the yield by decreasing the Phytopthora blight (*P. capsici* L.), using the seedlings of pepper (*capsicum annum* L) inoculated with *Glomus intraradices* vesicular arbuscular mycorrhizal (VAM) fungus in solarized area.

MATERIALS AND METHODS

Plant, pathogen and VAM fungus

The study was conducted in the field of Agricultural Faculty of Dicle University. The solarization was applied for two months (August-September) in 2007, and then lettuce plants with or without vesicular arbuscular mycorrhizal (VAM) were grown in this area in fall season of 2007. The same experiment area was also used for this research. Pepper (*Capsicum annuum* L. cv. Kandil) was used as a plant material. *P. capsici* Leonian isolates were obtained from West Mediterranean Research Institute and *G. intraradices* (VAM) was from the Soil Department, Agricultural Faculty, University of Çukurova.

Seedlings growing with VAM and non-VAM

Pepper seedlings were grown in controlled conditions. Torf was used as substrate and autoclaved for sterilization at 121°C for 90 min. Each viol included 45 eyes filled with sterilized torf, and mixture soil included VAM (15 g per each eye). Then this composition was covered with enough amount of torf. After then, pepper seeds were sown in the viols including infested mixed soil with VAM. Control treatments did not contain VAM.

Transplanting of seedlings

The pepper seedlings inoculated with VAM or not were transplanted to experimental area according to split-split-plot design. The experiment was established in 4 repetitions with 32 plots; solarized and non-solarized soil as main plots; VAM infested and non-VAM as sub-plots, which were inoculated with or without *P. capsici*. The same plots infested with VAM from the previous year were also infected with the same fungus (*G. intraradices*) used as VAM plot. The seedlings were transplanted in the field with 25x70 cm rows spacing and each plot includes 30 plants.

Preparation of inoculum

The isolate of *P.* capsici was obtained from West Mediterranean Agricultural Research Institute. This isolate was proliferated first on potato-dextrose agar (PDA) and then fungus was grown in erlenmeyers (250 ml) containing autoclaved wheat (800 g cracked wheat + 200 ml water). The grains were inoculated with *P. capsici* isolates and incubated at 22 ± 1°C in darkness for 3 weeks

Inoculation of soil with *P. capsici*

The grains including mycelial pieces of *P. capsici* were mixed with sand and inoculated to experiment area. The inoculum saturated with wheat grain of 125 g/m^2 dense was delivered in lines of 5 - 10 cm depth, and then mixed with soil. As soon as inoculation was completed, surface irrigation was applied. The lines in the control parcels, which are those without *P. capsici* inoculation, were mixed only with sand and then surface irrigation was applied.

Phytophthora blight incidence

After inoculation of *P. capsici*, disease symptoms were observed four times with a month's interval, from July to October. The observation was made after irrigation was done. Phytophthora blight disease incidence was rated according to visible symptoms included wilting of plants and plant mortality. We did not use any disease evaluation scale. Disease incidence was rated according to visible symptoms in the experiment area. Diseases incidence was transformed to angel value and variance analyses were conducted by using MSTAT-C programme.

Obtaing yield

Green pepper was harvested 16 times from June to November during the growing season in 2008. Unmarketable fruits were classified as exhibiting sunburn damage, blossom end rot, misshapen fruits, disease symptoms, and other defects. The total yield in each plot was calculated as yield per plant. The first five harvest yields were evaluated as early yields.

RESULTS AND DISCUSSION

Effect of *P. capsici* on yield and disease incidence

Damage caused by *P. capsici* was seen immediately, as visible symptoms including wilting of plants were followed by sudden death. At the first observation, mortality rate of plant was 8.9%, and then rose to 82.5% at the 4th observation in plots inoculated with *P. capsici,* in comparison to control plots (Table 1).

Incidence of Phytophthora blight diseases caused yield loss by 16.4% in the first five total harvests, and then this rate increased to 47.7% for a total of 16 harvests in plots inoculated with *P. capsici*. For both situations, the differences between applications were statistically significant at 1% level (Table 1).

Phytophthora blight caused by *P. capsici* was the most dangerous disease for affecting pepper cultivation in

Table 1. Effect of solarization and vesicular arbuscular mycorrhizal (VAM) on phytopthora blight (*Phytophthora capsici* Leonian) and yield in pepper (2008).

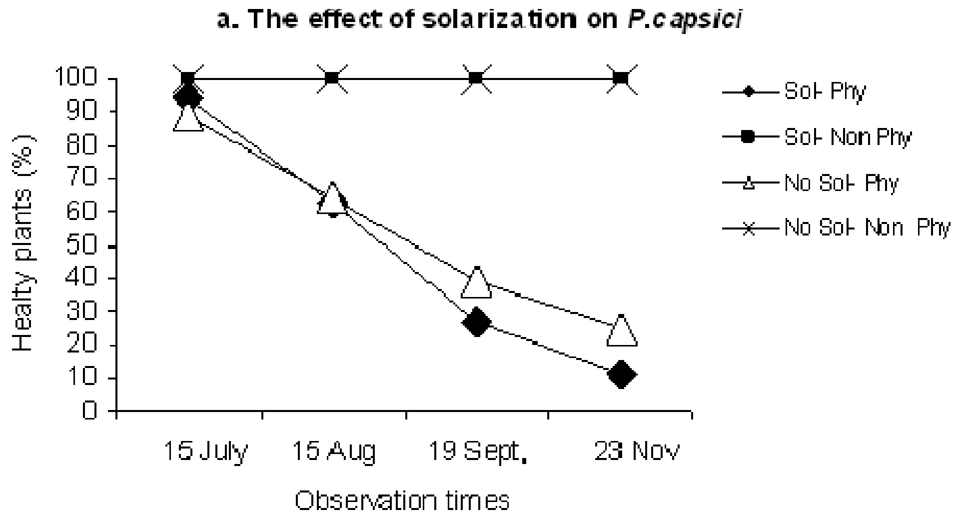
Treatments	Diseases incidence (%) (Observation times)				Total yield (g/plant)	
	First (15 July)	Second (15 Aug)	Third (19 Sept)	Fourth (23 Nov)	Total of 5 harvest (21 July)	Total of 16 harvest (26 Nov)
Solarized	3.12	18.95	36.87	44.58	215.00 a	752.31a
Non solarized	5.83	18.12	30.83	37.91	193.18 b	599.68 b
VAM	2.49 a	15.41	30.20	37.91	223.68 a	733.18 a
Non VAM	6.45 b	21.66	37.49	44.58	178.25 b	618.81 b
Sol- VAM	2.91 a	16.24	35.41	43.74	244.25 a	801.37
Sol- Non VAM	3.33 ab	21.66	38.33	45.41	185.75 b	703.25
Non Sol- VAM	2.08 a	14.58	24.99	32.08	203.12 ab	665.00
Non Sol- Non VAM	9.58 b	21.66	36.66	43.74	183.25 b	534.37
Inoculation of *P. capsici*	8.95	37.08	67.70	82.49	183.00 b	436.62 b
Non Inoculation of *P. capsici*	0	0	0	0	218.93 a	915.37 a
Sol- Phy	6.24	37.91	73.74	89.16	193.87	436.37 c
Sol- Non Phy	0	0	0	0	236.12	1068.25 a
Non Sol- Phy	11.66	36.24	61.66	75.83	172.12	436.87 c
Non Sol- Non Phy	0	0	0	0	214.25	762.50 b
VAM –Phy	4.99	30.83	60.40	75.83	207.12	510.00
VAM -Non Phy	0	0	0	0	240.25	956.37
Non VAM -Phy	12.91	43.33	75.00	89.16	158.87	363.25
Non VAM -Non Phy	0	0	0	0	210.12	874.37
Sol- VAM -Phy	5.83	32.49	70.83	87.49	223.00	487.25
Sol- VAM -Non Phy	0	0	0	0	265.50	1115.50
Sol- Non VAM -Phy	6.66	43.33	76.66	90.83	164.75	385.50
Sol- Non VAM -Non Phy	0	0	0	0	206.75	1021.00
Non Sol- VAM -Phy	4.16	29.16	49.99	64.16	191.25	532.75
Non Sol- VAM -Non Phy	0	0	0	0	215.00	797.25
Non Sol- Non VAM -Phy	19.16	43.33	73.33	87.49	153.00	341.00
Non Sol- Non VAM -Non Phy	0	0	0	0	213.50	727.75
Solarization	NS	NS	NS	NS	*	*
VAM	*	NS	NS	NS	**	*
SXVAM	*	NS	NS	NS	*	NS
P. capsici	**	**	**	**	**	**
SXP	NS	NS	NS	NS	NS	*
VAMXP	*	*	NS	NS	NS	NS
SXVAMXP	*	NS	NS	NS	NS	NS

* , ** Significant at 0.05 and 0.01 levels respectively. NS = not significant 0.05 level.

Turkey (Gocmen et al., 2004), and in the world at large (Ristaino and Johnston, 1999). It was reported that disease incidence could be higher than 85% with surface and frequently irrigation (Cafe-Filho and Duniway, 1995; Sagır et al., 2005).

Effect of solarization on occurrence of diseases caused by *P. capsici* and yield

At the beginning of the vegetative period, plant mortality caused by *P. capsici* in solarized plots was less than that



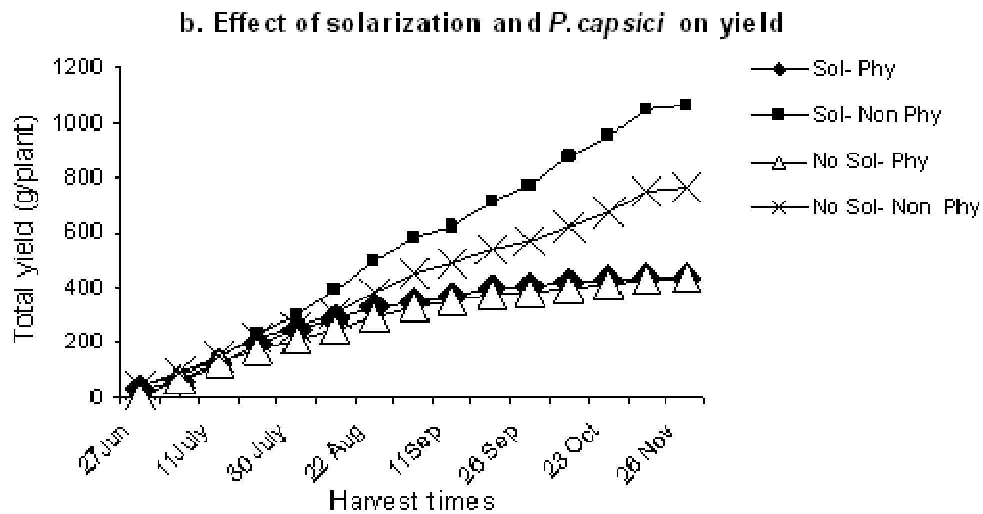


Figure 1. Effect of solarization on occurrence of diseases caused by *P. capsici* and yield.

in non solarized ones, but it was higher at the end of the experiment. In spite of this, the total yield increased to 20.94% by long term effect of solarization in second year, and effect of solarization on yield was significant at 0.05 level (Table 1 and Figure 1-a).

Interaction between solarization and *P. capsici* for fruit yield (per plant) was significant at 5% level in total harvest. While the highest yields were taken in parcel of "Sol+ non- *P. capsici"* (1068.25 g/plant) combination, the lesser yields were taken in both plots of solarized and non-solarized, inoculated with *P. capsici* (436.37 g/plant and 436.87 g/plant), respectively (Table 1 and Figure1-b). The increasing yield rate was 144% in solarized plots without inoculation of *P .capsici,* in comparison to inoculated ones.

The long-term effect of solarization is also continued in second and subsequent years, which was conducted in previous study (Katan et al., 1983; Candido et al., 2006). In such solarized or fumigated area, soil- borne pathogens like *P. capsici* will rapidly proliferate due to the existence of ecosystem brought by solarizatiıon effect, which leads to the decreasing or eradication of beneficial organisms (Gonzales et al., 2007).

Effect of VAM on occurrence of diseases caused by *P. capsici* and yield

Occurrence of Phytophthora blight diseases caused by *P. capsici* decreased in sub-plots inoculated with VAM,

Figure 2. Two-week-old pepper plants: (a) Occurrence of anthocyanin pigments in leaves of the pepper plant inoculated with VAM and (b) without **anhocyanin** pigments in leaves of the pepper plant non inoculated with VAM .

while it increased in non-inoculated VAM sub-plots. And this was higher in the beginning of plant development. When the parcels of soil having *P. capsici* inoculation were completed in a month, disease incidence caused by *P. capsici* was inhibited by 61.39% every 12 week in plants in sub-plots inoculated with VAM in comparison to control plots non-VAM ones under observation. This inhibition rate decreased with plant growth for the second, third and fourth observations respectively as: 28.85, 19.44 and 14.96% (Table 1).

The pepper plant may have become resistant to *P. capsici* because it must have taken enough nutrients from soil by means of increasing the root surface by the VAM. On the other hand, pepper might have acquired defense against *P. capsici* since phytoalexins may have been produced during early colonization of its roots by symbiotic *G. intraradices.* As a matter of fact, the capsidol known as phytoalexin among these chemicals, increased with inoculated + VAM in pepper (Ozgonen and Erkilic, 2007).

In our study, changing temperature in the first week of May, colonization of pepper roots by symbiotic *G. intraradices* was determined, as anthocyanin (color producing pigment) in leaves of pepper occurred in soil solarized plots with VAM inoculation. In contrast, occurrence of anthocyanin pigments in leaves of the pepper plants was not observed in plots without VAM inoculation (Figure 2 a-b). Anthocyanin occurrence may have been as a result of phytoalexin accumulation. However, there was no study reported or noted that any VAM fungi such as *G. intraradices* have caused anthocyanin occurrence in pepper. But, it was reported that some fungal pathogens had enhanced anthocyanin production in callus cultures of *Daucus carota* (Rajendran et al., 1994), and in addition to stress conditions had induced anthocyanin accumulation (Atanassova et al., 2001).

In the sub- plots of VAM, not only early flowering, but also fruit setting was observed (Figure 3 a-b). For the total five harvests, fruit yield was higher by 25.48% in sub-plots inoculated with VAM than in non-inoculated without VAM ones, and it was statistically significant at 0.01 level. This rate decreased to 19.20% in total of 16 harvests, and it was significant at 5% level (Table 1).

VAM fungus not only inhibited Phytophthora blight diseases caused by *P. capsici,* it also increased the yield at the beginning or end of growth season (Table 1 and Figure 4 a-b). The cumulative yield for the 16 harvests is arranged as the application of "VAM- Non- *P. capsici*", "Non -VAM-Non *P. capsici*", "VAM- *P. capsici*" and "Non VAM- *P. capsici*" combinations from high to low (Table 1 and Figure 4-b). Treatment of "VAM - Non *P.capsici*" that took place was higher by 163% in comparison to the least one, "Non VAM- *P. capsici*". The most important question that arose from the results was: why was the level of yield difference between the *P. capsici* infected plots treated with VAM and that without VAM application 40.39%? In previous studies, after VAM application, plant obtain more nutrients from soil by means of increasing the pepper root surface by VAM (Ortas et al., 2003), and parallel with this occasion, by decreasing Phytophthora blight disease, the yield increases (Ozgonen and Erkilic, 2007).

Effects of solarization and VAM fungus on disease Incidence of *P. capsici* and yield

The interaction effects of solarization and usage of VAM was found to be significant at 95% confidence interval in control of Phytophthora blight diseases caused by *P. capsici*, specifically at early development stage (Table 1).

Figure 3. Effect of VAM on early fruit settings; (a) Fruits on pepper plants inoculated with VAM, and (b) without fruit on pepper plants non inoculated VAM from the first harvest in solarized plots (Photo: 17 June, 2008).

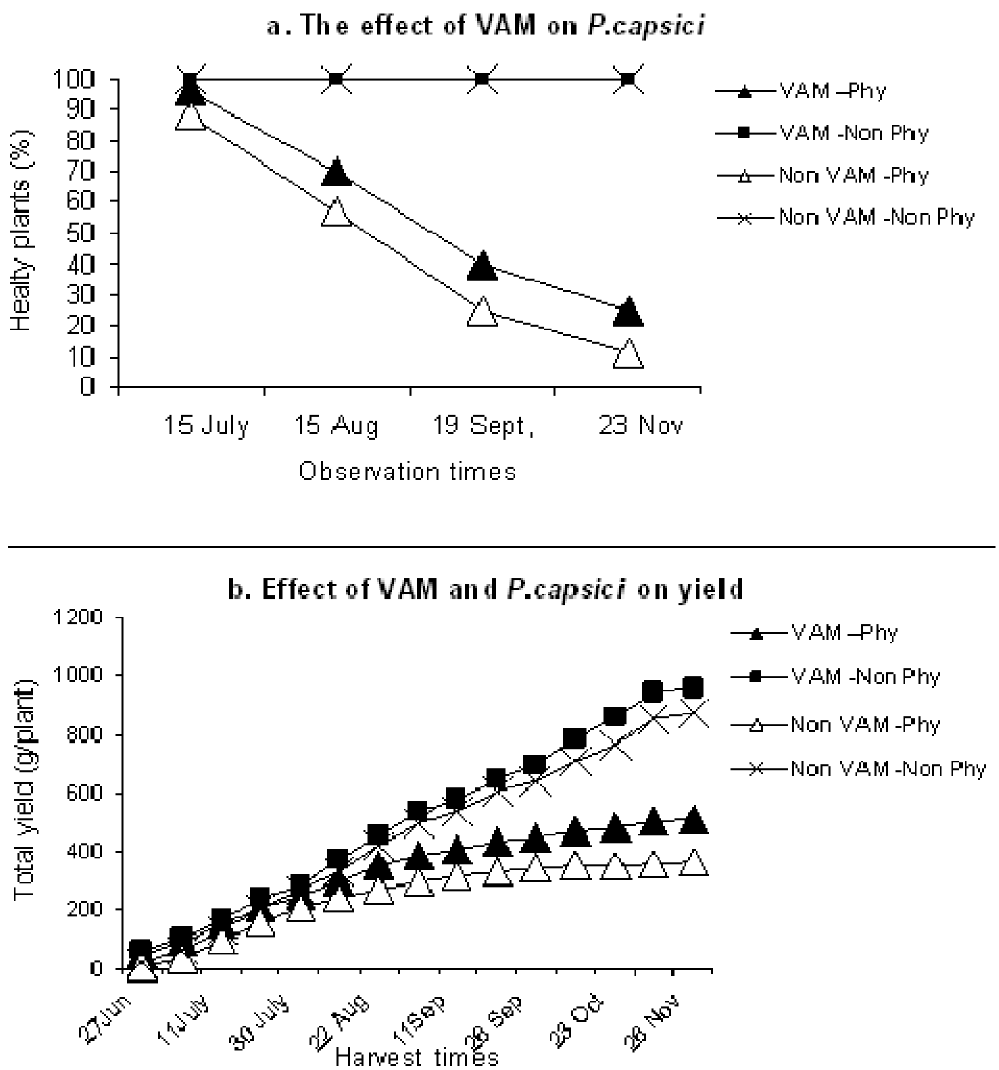
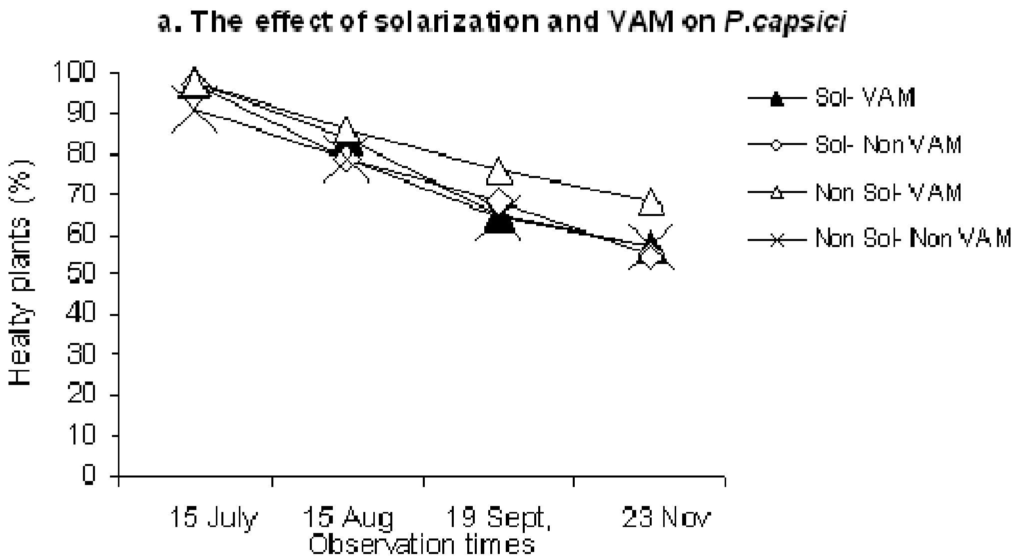


Figure 4. Effect of Vesicular Arbuscular Mycorrhizal (VAM) on occurrence of diseases caused by *P. capsici* and yield in pepper.



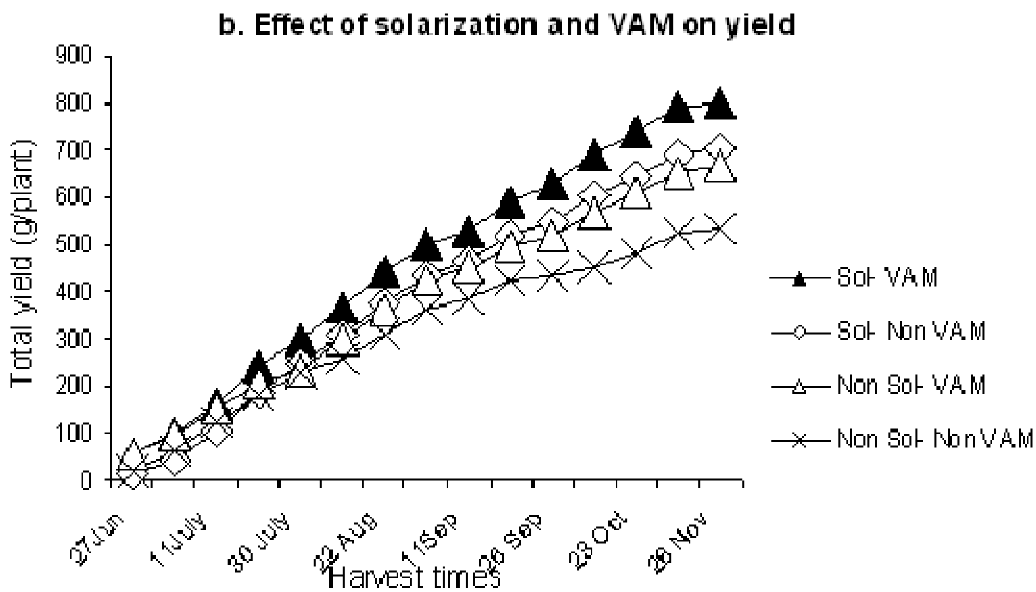


Figure 5. Effect of solarization with Vesicular Arbuscular Mycorrhizal (VAM) on occurrence of diseases caused by *P. capsici* and yield in pepper.

Less crop losses were recorded at the plots treated with VAM. The level of damage reduced by 78.28% between the Non- Sol-Non VAM (9.58%) (highest crop losses) plot in comparison to the lowest losses recorded plots No Sol-VAM (2.08%). The same trend was observed at the end of growing season. Again, the lowest level of dead plants was recorded at Non Sol-VAM (32.08%) that was replaced by Sol-Non VAM (45.21%). The control rate of diseases was 29.35% in this season (Table 1 and Figure 5-a)

The effects of solarization combined with VAM influenced fruit yield by interfering with the disease incidence. The highest yields were obtained at "solarization + VAM" plots where the lowest disease incidence was recorded, whereas there was the least yield in control plots without solarization and VAM, where the highest disease incidence was observed. The mean yields were significantly different ($P<0.05$) between combined solarization and plots treated with VAM and control plots in which neither solarization nor VAM was applied at early growing

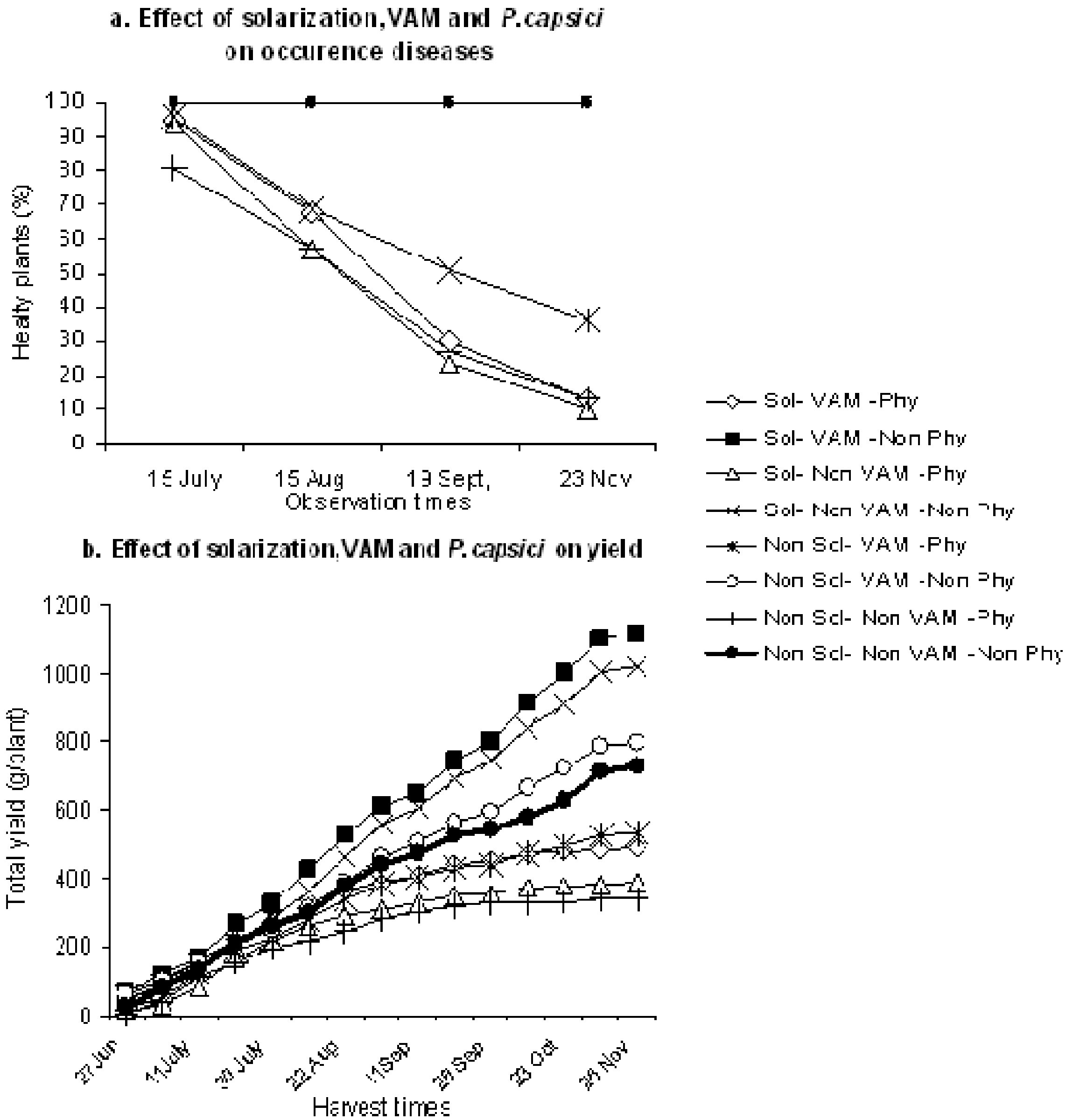


Figure 6. Effects of Solarization, Vesicular Arbuscular Mycorrhizal (VAM) and *P. capsici* fungus on occurrence with yield in pepper.

season. The difference level between these plots was 33.28%. This increment trend continued throughout the growing season and resulted in 49.96% increment at final assessment as total of 16 harvesting (Table 1 and Figure 6 b).

Natural microorganisms set up a balance in soils when solarization is not applied

Among these microorganisms, VAM was able to take place. When VAM was applied on soil artificially, densities of fungi increase. This means level of phytoalexins increase and result in a higher defense mechanism of plants since phytoalexins are an important component of defense. This may be the reason why high defense level was observed at early growth stage of plants when inoculated with the pathogen fungus, *P. capsici*. By increasing level of inoculums of *P. capsici,* this high defense mechanism caused by VAM decreased. 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 (Afek et al., 1991) and result in an

Figure 7. Effect of solarization, vesicular arbuscular mycorrhizal (VAM) and *P. capsici* on occurrence of diseases with plant growing in pepper: a. "Sol-Non VAM- *P. Capsici*"; b. "Sol- VAM- *P. Capsici*"; c. "Sol-Non VAM- Non *P. Capsici*"; d. "Sol- VAM- Non P. Capsici", (photographed on 15 August, 2008; before 8^{th} harvest).

increment in yield of pepper (Ortas et al., 2003).

Effects of interaction among solarization, VAM and *P. capsici* on yield

Effects of interaction among solarization, vesicular arbuscular mycorrhizal (VAM) and *P. capsici* on diseases and yield were given in Figures 6, 7 and 8. The highest total yield during vegetative season was obtained (1115,50 g/plant) in the combination of "Sol- VAM-Non Phy", and other ones followed this respectively: "Sol- Non VAM -Non Phy", "Non Sol- VAM -Non Phy", "Non Sol- Non VAM -Non Phy", "Non Sol- VAM -Phy", "Sol- VAM -Phy", "Sol- Non VAM -Phy". The lowest yield was 341.00 g/plant obtained in "Non Sol- Non VAM -Phy" (Table 1 and Figure 6-b). The increase of yield rate in plot of the highest yield obtained was 227% higher than the ones with the lowest yield. Together, application of solarization and VAM in the plot infested by *P. capsici*, the increase of yield rate was 42.83% higher than those without both of them in the plot infested by *P. capsici.*

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Figure 8. Effects of Vesicular Arbuscular Mycorrhizal (VAM) and *P. capsici* on occurrence diseases with plant growing in pepper. a. "Non Sol- Non VAM - *P. capsici*", b. "Non Sol- VAM - *P. capsici*", c. Non Sol- Non VAM -Non *P. capsici*", d. "Non sol- VAM -Non *P. capsici*" (photographed on 15 August, 2008; before 8th harvest

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