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Effect of Soil Solarization with Amendments to Soil-born Fungal Pathogens and Yield in Strawberry Production

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ABSTRACT

Field experiments were carried out in a high tunnel strawberry field in Sultanhisar town of Aydin province during 2004 - 2005 cropping season. Plot size was 4.5 X 40 m for each character. Organic amendments used including winter growing broad bean, cabbage, cauliflower, broccoli and wild turnip plants were incorporated into soil by harrowing and sulfur dust was also applied at two different levels, 500 kg ha^{-1} (sulfur I) and 1000 kg ha^{-1} (sulfur II). Six-week soil solarization was applied to all plots after preparing planting beds. Following solarization, strawberry seedlings (cv. Camarosa) were planted at the first week of August. For bacterial treatments, strawberry seedlings were dipped into the bacterial suspension (10^8 cell ml^{-1}) of *Serratia plymuthica* (HRO-C48) and fluorescent *Pseudomonas* (4K1) isolates before planting. One month later bacterial treatments were reapplied by drenching 10 milliliter of the same bacterial suspensions into soil around each plant.

There was significant difference among the treatments and, the highest yield (46820 kg ha^{-1}) was obtained from broad bean treatments. Other treatments resulted in the following yield values; broccoli 46300 kg ha^{-1}, cauliflower 45370 kg ha^{-1}, cabbage 43680 kg ha^{-1}, only soil solarization 42980 kg ha^{-1}, 4K1 41700 kg ha^{-1}, radish 41190 kg ha^{-1}, HRO-C48 39720 kg ha^{-1} sulfur I 34560 kg ha^{-1}, and sulfur II 31800 kg ha^{-1}. The lowest percentage of dead strawberry plants was observed with solarization only plots (26%). This was followed by broad bean (27.7%), broccoli (31.3%), cauliflower (32%), cabbage (35.3%), HRO-C48 (36.7%), radish (38.6%), 4K1 (43.5%), sulfur II (52.6%) and sulfur I (56.2%) treatments.

Key words: Biofumigation, broad bean, *Brassica* spp., sulfur, *Serratia*, *Pseudomonas*.

INTRODUCTION

Aydin province is a major strawberry growing region supplying 60% of strawberries in Aegean region, which amounts to 8.4% of the strawberries grown in Turkey. Sultanhisar, a town in Aydın province, supplies 92% of total strawberry production from the province, which amounts to 12,320 (Anonymous, 2005; FAO, 2006). Strawberries grow best in pH of 6 to 6.8 (Zeiley and Shry, 2004). The soil pH is generally high and tending to be too alkaline (>7.0) in the strawberry growing areas of Aydın. Therefore, strawberry growers have to take some measures to reduce soil pH and they usally add sulfur into the soil. Due to extended use of high tunnels and same growing fields, soil borne pathogens specifically *Phytophthora cactorum* (Lebert and Cohn) J. Schröt., and *Rhizoctonia solani* Kühn. were shown to cause significant loses (Benlioğlu *et al*., 2004; Benlioğlu *et al*., 2005). Therefore, control of soil borne pathogens is an important topic in strawberry fields.

The most effective method to control soil-borne fungi is soil disinfection. There have been increased efforts in methyl bromide (MeBr) replacement in Turkey focused their attention to solarization or the combination of low dose metam sodium and short term solarization have been noted as the most effective control method (Benlioğlu *et al*., 2004; Benlioğlu *et al*., 2005). Benlioğlu *et al*., (2004) stated that solarization increased the yield after the first year by 163.3% and second year 27.8% compared the control plot. The dead plant count also increased after the first year by 10%, and second year 83.8% in control. Among disinfection methods, solarization is the most preferred method by the farmers due to its low cost, reusability of polyethylene sheet, and direct impact on marketable strawberry yield. It is reported that strawberry is one of the most receptive plants to disinfection with up to 35% increased yields (Porter and Mattner, 2002). Interestingly, solarization causes larger reduction in pathogenic fungi, and bacteria, otherwise stimulation of beneficial microorganisms. Moreover, their population increases rapidly after solarization (Katan, 1981; Elmor *et al*., 1997).

Soil solarization alone may not be consistently effective for the control of soil-borne pathogens. In such cases, soil amendments have been used to enhance the performance of solarization (Ramirez-Villapudua and Munnecke, 1988; Gamliel and Stapleton, 1993a). An important emerging method called “biofumigation” is incorporating biologically active plants into soil. Such treatments enrich organic matter content of soil and increase yield. Many plants from Brassicaceae including cabbage, cauliflowers, broccoli, and Poaceae synthesize glucosinates (GSL), which is degraded to methyl isothiocyanide (MITC), and ally isothiocyanide (AITC). MITC is known to have anti fungal effect on soil borne fungi. MITC is also a byproduct of metam sodium, which is a known fumigant. Such secondary products (GSLs) are commonly found in tissues of brassicaceae members; however, their concentrations vary from plant to plant. Their concentration and fungicidal effects are directly proportional (Gamliel and Stapleton, 1993a; Smolinska *et al*., 1997; Tsao *et al*., 2002). In laboratory and field trials to determine the effects of incorporating shredded brassica tissues into soil, it was found that such attempts resulted in up to 100% reduction in *V.dahliae* microsclerotia density (Parker *et al*., 2005). In another study, *Phytophthora cactorum* population was 10% lower in biofumigated fields compared to those that are resting and there was 30% reduction in weed population (Bianco *et al*., 2000).

Another common treatment is combination of solarization with biological agents such as *Trichoderma* spp., *Talaromyces flavus*, *Bacillus* spp., *Pseudomonas* spp. (Elmor *et al*., 1997; Subbarao *et al*., 1999). A study done in Israel found that fluorescent *Psuodomonas* population was 6-10 times higher in lettuce rhisozphere grown in solarized soil compared to non-solarized controls (Gamliel and Stapleton, 1993b). Higher population densities of *Bacillus* spp. and fluorescent *Psuodomonas* in solarized soil were suggested to play a critical role in pathogen depression and higher yield (Stapleton and Devay, 1984; Gamliel and Katan, 1991).

This study was carried out to determine the effect of combinatorial treatments including biologically active plants, antagonist bacteria, and sulfur with solarization on soil-borne pathogens and strawberry yield.

MATERIALS and METHODS

Field sites, experimental design and statistical analysis

In 2004–2005 cropping seasons, field experiments were performed on a loamy sandy soil in a commercial field in Sultanhisar, Province of Aydın, has a history of strawberry production and diseases pressure from *Rhizoctonia solani* and *Phytophthora cactorum*. Prior investigations confirmed that *Rhizoctonia solani* and *Phytophthora cactorum* were present and causing disease (Benlioğlu *et al*., 2004). Experimental plots consisted of four adjacent beds measuring 4.5 X 40 m, arranged in a randomized block design with four replications per treatment. Also plots were separated within beds by 1.2 m buffer areas at each end of the plot.

The strawberry seedlings used in this study were frigo seedling of cv. Camarosa. Data were first analyzed by ANOVA, followed by mean separation using Fisher's protected least significance difference test. All analysis was performed with the JMP IN program at P=0,05.

Treatments

Organic amendments were selected from locally available and commercially grown broad beans (*Vicia faba*), cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*Brassica oleracea* var. *botrytis*), broccoli (*Brassica oleracea* var. *italica*), radish (*Raphanus sativus*) cultivars. *Serratia plymuthica* (strain HRO-C48 kindly provided by Gabriel Berg, University of Rostock, Germany), one *Pseudomonas putida* (strain 4K1) (Özyilmaz, 2008) and sulfur dust were also used.

For the organic amendment treatments; broad beans (20 plant m^{-2}), cabbage (4 plant m^{-2}), cauliflower (4 plant m^{-2}), broccoli (4 plant m^{-2}) and radish (60 plant m^{-2}) were planted in November 2003 in the experimental plots. All plants were incorporated into soil as green manure by harrowing and ploughing in March 2004. Above mentioned biologically active organic amendment residues were tilled into soil in March 2004 at the end of harvest of commercial field production. On the other hand sulfur dust used at two different levels at 500 kg ha^{-1} (sulfur I) and, 1000 kg ha^{-1} (sulfur II), was mixed into soil with a rototiller and covered PE sheet at the same time of solarization. Soil solarization was applied on all experimental plots including un-amended controls. For this purpose, raise beds were built, drip lines were laid in each plot and they were covered with transparent plastic sheet (0.1 mm thick) on June 28, 2004. Solarization was lasted after six weeks on August 3, 2004. Soil temperatures at 0, 10, 20 cm deep were measured at hourly intervals using Hobo data loggers (Onset Computer Corporation, USA) and the highest temperatures values (at 16 pm) were used for comparison in different depths of soil during solarization period (6 weeks).

Strawberry seedlings were planted on August 5, 2004. Plants were placed in raise beds on a straight line with 30 X 30 distances in between.

The antagonistic bacteria were grown for 48 h at 25 °C on tryptic soy broth (Difco) and pelleted by centrifugation (5000 g, 5 min, 4°C). Bacteria was resuspended in phoshate buffer (0.01M pH=7.2) and diluted to a concentration of 10^8 cell ml^{-1}, and were used immediately for treatments.

Before planting, strawberry seedlings was first dipped into bacterial suspension for 15 min and then planted. As a part of bacterial treatments within one month of original application, bacterial solution prepared the same way was applied at a volume of 10 ml $plant^{-1}$ by soaking the soil around them. The black PE mulch was used cover the raise beds in late September. Throughout vegetation, plants were monitored and normal cultural procedures were followed and no fungicides treatments against soil borne pathogens.

Disease Incidence: To determine the effect of treatments on soil borne diseases, which strawberry plants showing poor growth and typical root or crown rot symptoms or dead plants were counted and disease incidence was calculated as percentage of infected plants related to total plants per plots. First count was done about 2 months after planting on 04.11.2004 and second count was after one month following the last harvest on 22.06.05. Ten randomly dead plants from each plot were carefully dislodged, and examined for root and crown rot symptoms by cutting through the crown longitudinally. Based on the initial observations, small pieces of necrotic root or crown tissue was excised aseptically and placed on potato dextrose agar or corn meal agar supplemented with antibiotics (Duncan *et al*., 1987). After incubation in an incubator at 21ºC for 3–7 days, cultures were characterized and identified microscopically.

Yield measurements: Throughout harvesting season, fruits were harvested at least once every week for the marketable strawberry yield was recorded from 5 April to 22 June 2004, for each experimental plot.

RESULTS

Soil temperature: Soil temperatures registered at 4 pm are shown in Figure 1. Soil temperatures on the surface were measured generally to be higher than 70 ºC, with maximum and minimum temperatures being 76.8 and 56 ºC, respectively. On the other hand 10 cm below the surface, temperatures were measured to be around 50ºC and maximum temperature were 51.8 ºC and minimum temperature were 45.9ºC. Temperatures at 20 cm depth were around 40 ºC and maximum and minimum soil temperatures were registered to be 41.5 and 38.3 ºC, respectively. As a result, at a common root depth of 20 cm, soil temperatures remained mostly above 40 ºC as shown in Figure 2.

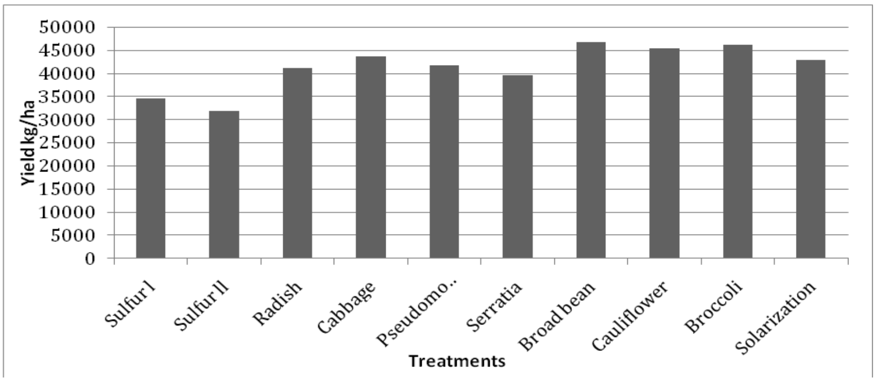

Figure 1. Mean marketable strawberry fruit yield (kg/da) for various treatments during 2004-2005 cropping season, Values at the same column with different letters show significant differences at P=0.05 according to the Fisher's least significant difference (LSD) test.

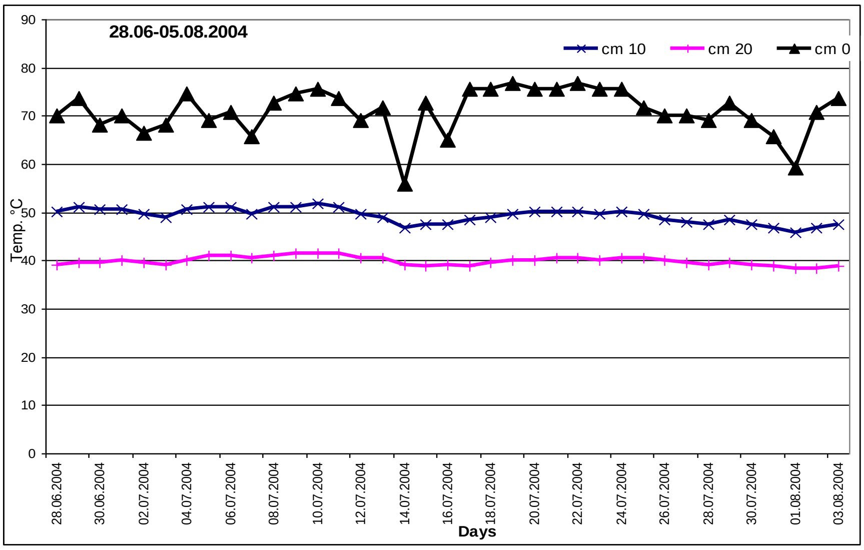

Figure 2. Soil temperatures on the surface, 0, 10 and 20 cm below surface registered throughout solarization.

Effects on soil borne pathogens: Isolations from dead plants indicated that *R. solani* was the only pathogen in both counts during 2003-2004 cropping season. The highest loses were observed with sulfur treated plots in the first count. Neither dosage of sulfur made a difference. Other treatments were not statistically different in the first count. In the second count, the highest loses were again observed with sulfur treatment. The lowest loses were observed with solarization alone, solarization broad bean, broccoli, and cauliflower combinations.

Against soil borne pathogens *S. plymuthica* (HRO-C48), one of the antagonistic bacteria, treatment was statistically clustered with solarization alone, and solarization + amendment treatments. On the other hand *Pseudomonas sp*. (4K1) was clustered with sulfur treatments. Results are shown in Table 1.

Table 1. Percentage of dead strawberry plants obtained from various treatments during 2004-05 cropping season

Characters	**Number of Plants**				
	Total	**1st count**		**2nd count**	
		Dead	**Incidence %**	**Total Dead**	**Incidence %**
Sulfur I	291,5	82,3	28.2 A	163,8	56.2 A
Sulfur II	261,5	67,3	25.7 A	137,5	52.6 AB
Radish	290,5	44,3	15.2 B	112,3	38.6 BCD
Cabbage	293,3	44,5	15.2 B	103,5	35.3 CD
Pseudomonas	262,3	38,8	14.8 B	114	43.5 ABC
Serratia	283,8	41	14.4 B	104,8	36.7 BCD
Broad bean	259,8	36,8	14.2 B	72	27.7 CD
Cauliflower	258,5	32,8	12.7 B	82,8	32.0 CD
Broccoli	258	30,8	11.9 B	80,8	31.3 CD
Solarization	260,5	41,8	16.0 B	67,8	26.0 D

(*)Values in the same column with different letters show significant differences at P=0.05 according to the Fisher's least significant difference (LSD) test. Data are means of four replicates.

Effect on plant yield: The total marketable fruit yields in the cropping seasons are presented in Table 1. Several treatments resulted in yields higher than 4000 kg ha^{-1}. There were statistically differences among the treatment. However organic amendment improved the yield and highest marketable strawberry yield was attained with broad bean/solarization treated plots at a yield of 46820 kg ha^{-1}. This was followed by solarization combinations with broccoli (46300 kg ha^{-1}), cauliflower (45370 kg ha^{-1}), cabbage (43680 kg ha^{-1}) and solarization alone (42980 kg ha^{-1}). As shown in Table 1, yields in plots treated with sulfur I and II treatments were 20 and 27% lower than solarization alone. On the other hand plots treated with *Serratia*, *Pseudomonas* and radish did not increase yield compared to solarization treatments alone.

DISCUSSION

We obtained the highest temperatures during solarization period (6 weeks) at 10, 20 cm below the surface were measured to be around 50, 40 ºC, respectively. As a result of our observations, at a strawberry effective root depth of 20 cm, soil temperatures remained mostly above 40 ºC and these temperatures effectively controlled soil-borne fungi. Devay and Katan (2000) have shown that the major portion of fungal pathogens was killed at 37ºC, within 2 to 4 weeks. On the other hand, 47ºC was sufficient to kill 90% of the pathogens within 1 to 6 hours.

Bio-fumigation has the potential to significantly contribute to control of soil-borne pathogen and yield. Several studies show that incorporation of organic amendments increased effectiveness of solarization (Stapleton *et al*., 2000; Gamliel and Satapleton, 1993b; Benlioğlu *et al*., 2005). However, results from some other groups somewhat differed. No positive impact of rotation of strawberry, broccoli, brussels sprout and rye on Verticillium wilt on fields contaminated with *Verticillium dahliae* was reported (Duniway *et al*., 2000). Indeed, similar results were observed in our study, organic amendments, and sulfur did not enhance the effect of solarization in terms of disease severity. However positive effects of organic amendments were observed on the yield. Higher yields were also observed with rotation of strawberry with radish (*Brassica jounce* L), and arugula (*Eruca sativa* Mill (cv Nemat) when compared to controls and classic green fertilizer applications (Lazzeri *et al*., 2003). Based on the recent studies in Australia, it was noted that 10% of strawberry growers, 10% of strawberry seedling growers and some strawberry onion farmers use bio-fumigation and combinatorial treatments (Bianco et al., 2000).

We used elemental sulphur (500 and 1000 kg/ha) to lower pH in our two experimental plots before solarization. Erdal et al., (2006) reported that S application to strawberry field corresponding to 500 and 1000 kg/ha decreased the soil pH from 8.3 to 7.9 and 7.7, respectively. In our experiment, application of elemental S caused to lower soil pH, but this was resulted in the highest plant loses in the sulfur treated plots. It is known that soil bacteria particularly *Thiobacillus* spp., convert the sulfur to sulfuric acid lowering the soil pH (Kuenen, 1975). This process occurs when the bacteria are active, when the soil is moist and warm. The soil temperature needs to be above 13°C. The bacteria are not active in the winter so fall applications of sulfur have no affect on the soil pH next spring. However, when the soil is too wet, the sulfur is converted to hydrogen sulfide (H_2S) by anaerobic bacteria, and H_2S can kill the roots of plants (Cox and Koeing 2003). The high moisture in our S amended plots due to the solarization procedure could be reason for high plant mortality in S treated plots.

In this study, antagonistic bacteria also were used to against on soil-borne pathogenic fungi. However, these antagonistic bacteria did not enhance the effectiveness of solarization in terms of the yield and disease severity. This is in disagreement with the reports on the beneficial effects of treatments with *Serratia* and

Pseudomonas spp. Eayre (2000) reported higher strawberry yields when treated with rhizobacteria (PGPR) 130 isolate, *Serratia marcescens* Auburn 90-166, *Pseudomonas putida* Ecoscience A8C59 and *Bacillus* spp. Gustafson varieties. Kurze *et al*., (2001) reported that strawberry yield was higher than 60% compared to control when strawberry seedlings were dipped into spore suspension of *Serratia plymuthica* HRO-C48 prior to planting. Furthermore, disease severity caused by *Verticillium dahliae* and *Phytophthora cactorum* decreased 24.2 and 9.6% respectively.

In Aydın province, strawberry has been grown same fields for many years without rotation. To avoid shortcomings this policy, it is very important to consider rotation. Most suitable candidates include biologically active plants that also have high market value. These plants can also be tilled into soil after harvesting to enrich the soil. And also soil solarization provides of infested soil economic control of many soil-borne pests and weeds, enhances the physical and chemical properties of the soil, and increases the yield of subsequent crops. Furthermore, the use of solarization along with biologically active plants incorporated into soil can help achieve better disease control and improve the yield, especially in organic farming.

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ÖZET

ÇİLEK ÜRETİMİNDE SOLARİZASYON VE ORGANİK MADDE UYGULAMALARININ TOPRAK KAYNAKLI FUNGAL PATOJENLERE VE VERİME ETKİSİ

Denemeler, 2004-2005 üretim sezonunda Aydın ili Sultanhisar ilçesinde yüksek tünelde ticari çilek üretimi yapılan bir tarlada her karakter için 4.5 X 40 m büyüklükteki parsellerde yürütülmüştür. Organik madde olarak kışın ekimi yapılan bakla, lahana, karnabahar, brokoli ve turp bitkileri tarla sürülerek toprağa karıştırılmıştır. Kükürt uygulamalarında ise dekara 500 kg ha^{-1} (kükürt I) ve 1000 kg ha^{-1} (kükürt II) olmak üzere toprağa toz kükürt ilave edilmiştir. Tüm parsellerde masuralar oluşturulduktan sonra 6 hafta süreyle solarizasyon uygulanmıştır. Çilek fideleri (Camorosa çeşidi) solarizasyon sonrası ağustos ayının ilk haftasında dikilmiştir. Bakteri uygulaması yapılan parsellerde fideler öncelikle *Serratia plymuthica* (HRO - C48) ve fluoresan *Pseudomonas* sp. (4K1) bakterilerinin 10^8 hücre ml^{-1}'lik süspansiyonlarına batırıldıktan sonra dikilmiştir. Bakteri uygulamasından yaklaşık 1 ay sonra aynı şekilde hazırlanan bakteri süspansiyonları bitki başına 10 ml olacak şekilde toprağa içirme şeklinde tekrar uygulanmıştır.

En yüksek verim bakla uygulanan parselden 46820 kg ha^{-1} olarak alınmıştır. Bunu sırasıyla brokoli (46300 kg ha^{-1}), karnabahar (45370 kg ha^{-1}), lahana (43680 kg

ha^{-1}), tek başına solarizasyon (42980 kg ha^{-1}), 4K1 (41700 kg ha^{-1}), turp (41190 kg ha^{-1}), HRO-C48 (39720 kg ha^{-1}), kükürt I (34560 kg ha^{-1}) ve kükürt II (31800 kg ha^{-1}) uygulamaları izlemiştir. Hasta bitki sayılarına göre uygulamalar değerlendirildiğinde en az hastalıklı bitki oranı tek başına solarizasyon uygulamasından (%26) elde edilmiş, bunu sırasıyla bakla (%27,7), brokoli (%31,3), karnabahar (%32), lahana (%35,3), HRO-C48 (%36,7), turp (%38,6), 4K1 (%43,5), kükürt II (%52,6) ve kükürt I (%56,2) takip etmiştir.

Anahtar Kelimeler: Biyofümigasyon, bakla, *Brassica* spp., kükürt, *Serratia*, *Pseudomonas*.

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