Applied of soil solarization for control of Egyptian broomrape (*Orobanche aegyptiaca*) on the cucumber (*Cucumis sativus*) in two growing seasons (in Iran)

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Broomrape or Broom-rape (Orobanche) is a genus of about 150 species of parasitic herbaceous plants in the family Orobanchaceae, mostly native in the temperate regions. Cucumber cultivation in the Middle East and Mediterranean region is susceptible to infestation by the parasitic weed Egyptian broomrape (Orobanche aegyptiaca), and resulting in severe yield losses. The effectiveness of solarization, a soil disinfection technique that uses passive solar heating, to control the incidence of broomrape under greenhouse conditions was studied over two growing seasons. Solarization was accomplished by the application of clear polyethylene sheets to moist soil for 63 days during the hot season. The treatment increased maximum soil temperature by around 15 °C, and at 5 cm below the soil surface, a temperature of more than 45 °C was reached for 34 to 60 days whereas this temperature was not reached at all in the first season and not for 20 d (second season) in unmulched soil. In solarized soil, no broomrape shoots emerged, and neither haustoria's nor underground tubercles of the parasite were found on cucumber roots. The treatment killed about 95% of buried viable seed, and induced secondary dormancy in the remaining 5%. In nonsolarized plots, broomrape shoots were still present at a high density, decreasing the plant growth and fruit production. Fruit yield was 33 to 88% higher in the solarized as compared with the nonsolarized treatment. Based on the results, we suggested that soil solarization, with precludes chemical contamination and are suitable for organic farming, is an appropriate technology where in the risk of Egyptian broomrape infestation is high.

Key words: Egyptian broomrape, (*Orobanche aegyptiaca* L.) cucumber, (*Cucumis sativus*), greenhouse production, cucumber yield, soil solarization, soil temperature, seed viability

Introduction

Broomrapes (*Orobanche* spp) are parasitic weeds that cause serious damage to several crops in Europe, the Mediterranean region, Central Asia, the

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Arabian Peninsula and some African countries (Klein and Kroschel, 2002). The life cycle of most parasite occurs belowground connected to the host plant and therefore, it's very difficult to control by either agronomic practices or herbicides (Goldwasser et al., 2003). In addition, control is made difficult by the fact that each plant produces thousands of dust-like seeds of 0.2- to 0.3-mmdiam that are readily distributed and remain viable in the soil for many years (Goldwasser et al., 2001). Although a broad spectrum of control methods has been attempted (cultural, physical, chemical, and biological), no significant reduction of infestation has been achieved. Hence, other control methods are needed (Klein and Kroschel, 2002). Among the Orobanche species, Egyptian broomrape (Orobanche aegyptiaca L.) is one of the most prevalent and devastating. Together with Egyptian broomrape (Orobanche aegyptiaca Pers.), it infests about 2.6 million ha of solanaceae crops (primarily tobacco, potato, tomato, cucumber, and eggplant) and Cucurbiaceae family (cucumber), mainly in the Mediterranean basin, North Africa, and Asia (Boari et al., 2003; Qasem, 1998). In certain parts of Greece, Israel, Lebanon, and Jordan, Egyptian broomrape has frequently damaged tomato, tobacco, and potato fields, with 100% yield losses having been recorded (Goldwasser et al., 2001; Haidar et al. 2003; Lolas, 1994; Qasem, 1998). Other crops in the Mediterranean region commonly parasitized and severely impacted by Egyptian broomrape include sunflower, oilseed rape, cucumber and carrot. It has become a recognized agronomic threat in western France (Gibot-Leclerc et al., 2004) and has been recorded for the first time in Australia, Central America, and the United States (Boari et al., 2003). In Italy, branch broomrape and Egyptian broomrape is responsible for significant yield losses in tobacco, cucumber, cabbage, and tomato (Boari and Vurro 2004; Boari et al., 2003; Diana and Castelli, 1994) and has caused damage to both field and greenhouse cucumber crops in southern Italy. Cucumber is the main field and greenhouse vegetable crop of the coastal areas of the Mediterranean Basin and Middle East. In Iran, in open field, cucumber occupies an area of 87,000 ha and produces about 4,00 Mkg of fruit, whereas greenhouse production occurs on about 4,200 ha. Pathogen, insect, and weed management in the greenhouse relies on regular preplant soil fumigation with methyl bromide, which also controls Egyptian broomrape. However, the use of this fumigant is compromised by its destructive effect on atmospheric ozone (Noling and Becker, 1994). As a result, they scheduled to phased out between 2005 and 2015, according to the revised Montreal Protocol. In the European Union, use of methyl bromide as a soil, commodity, and structural fumigant has been banned since January 1, 2005 (European Regulation CE 2937/2000). Therefore, there is an urgent need to identify alternative technologies for broomrape control. In southern Italy and other Mediterranean countries, an attractive alternative method is soil solarization (Katan 1987; Mauromicale *et al.*, 2001, 2005; Stapleton and DeVay, 1986), alone or in combination with other methods. This approach has attracted the interest in many warm-climate countries because of its effectiveness, simplicity, and safety for humans, plants, and the environment (DeVay and Katan, 1991; DeVay and Stapleton, 1997). Solarization entails covering wet soil with transparent polyethylene sheets during the hot season (Katan *et al.*, 1976). This serves to trap solar energy, thereby, heating the soil sufficiently to destroy soil pests. The temperature increase achieved is primarily in the result of the elimination of evaporation, but is also partially because of the greenhouse effect created (Mahrer, 1979). Its effectiveness has been demonstrated in many countries around the world (Katan, 1991). Soil solarization has been proven to be the most effective methods in controlling broomrape in open crops fields (Haidar and Sidahmad, 2000; Jacobsohn *et al.*, 1980; Mauromicale *et al.*, 2001; Sauerborn *et al.*, 1989).

The main objective of this research was to evaluate the effectiveness of soil solarization for the control of Egyptian broomrape in heavily and naturally infested greenhouse soils in southern Italy and for the improvement of plant growth and fruit yield in the cucumber crop. The effect of soil solarization on germination and viability of buried Egyptian broomrape seeds was also examined.

Materials and methods

Site, climate and soil

During 2005 to 2006, the seasons on the coastal plain south of Hashtgerd (Tankaman), (lat 35 50 ' N, long 50 58 ' E, 112.5 m above sea level) in a moderately deep Calcixerollic Xerochrepts soil naturally infested with egyptian broomrape. Before solarization, the soil characteristics were 17.2% clay, 27.1% silt, 53.8% sand, and 2.3% organic matter with pH 7.4, 1.6 % total nitrogen (N), 120 ppm available phosphorus (P), and 700 ppm exchangeable potassium (K). The local climate is like semiarid/Mediterranean with mild winters and hot, rainless summers. The mean 30- yr maximum summer monthly temperatures are 30.6 °C (June), 35.2 °C (July), 34.6 °C (August), and 29.7 °C (September).

Experimental design and soil solarization

In both seasons, in the experiments were arranged in a completely randomized design with two treatments as: solalized and non-solarized soils. The experiments were repeated your times. The plot size was 3 m by 15 m. During late spring, the soil was ploughed several times to provide a uniform surface and then leveled. One day before mulching, the soil was irrigated in field capacity because solarization is more effective with moist soil (Katan, 1981) because of the increased in thermal sensitivity of resting structures and improved heat conduction. Solarized plots were covered with 30-mm-thick transparent polyethylene film (\geq 88% total visible transmittance and 25% infrared radiation [IR] absorption) from July 10, 2005, to September 8, 2005, and from July 8, 2006, to September 11, 2006. Nonsolarized soil was similarly tilled and watered. The other weeds were irradicate by hand weeded.

Soil temperature measurement

During solarization period, the soil temperature was recorded continuously (every 30 min) at 5 and 15 cm depths, using thermistors buried in the center of the plots (solarized and nonsolarized) and connected to a portable digital microprocessor.

Plant material and management practices

Five-week-old cucumber transplants (Nasim cultibar) were planted in October 10, 2005, and October 15, 2006, with a within-row planting distance of 0.4 m, and an interrow spacing of 1.15 m; this gave a density of 2.2 plants m^2 . As the plants grew, lateral shoots were manually removed, and the resulting single stem was trained to the highwire system. Plants were headed back after twelve trusses at 120 (first season) and 135 (second season) days after planting (DAP). A fertigation program, commonly used in the area for cucumber crops, was adopted (34.4 g N, 228.5 g phosphorus pentoxide $[P_2O_5]$, 30.2 of potassium oxide [K₂O], 25.4 magnesium oxide [MgO], and 0.5 g iron [Fe] per season per cucumber plant). Drip irrigation was carried out when the accumulated daily evaporation reached 25 mm, 100% of maximum evapotranspiration (ETP). Pest was controlled using standard commercial practice. Bumblebees (Bombus *terrestris* L.) were introduced into the greenhouse to improved pollination. The oldest cucumber plant leaves were periodically removed. Harvesting was carried out from January 16, 2005, to May 6, 2005 (106 to 213 DAP), and from January 28, 2006, to May 15, 2004 (113 to 220 DAP). No additional heat, light, or carbon dioxide (CO_2) was provided in the greenhouse during the crop cycle.

Seed germination and viability

Mature Egyptian broomrape seeds were collected in May 2004 at the same location as the experiment, and 10-mg subsamples were stored in the dark

at room temperature (18 to 25 °C). Seeds were placed in 0.15-mm by 0.15-mm finemesh, nylon gauze bags, and on the day before the treatment initiation, bags were buried at 5 and 15 cm depth in each plot. One day after removing the polyethylene covers, all bags were recovered from the soil, the Egyptian broomrape seed was removed, and germination and viability tests were carried out. For the germination test, 250 seeds were placed in a 9-cm Petri dish on a piece of filter paper (Whatman No. 33) with 5 ml of stimulant solution and incubated in the dark at 18-61 °C. The stimulant solution was prepared by dissolving 10 mg of the synthetic germination stimulant GR242 (Johnson *et al.*, 1981) in 10 ml of acetone and diluting 1,000-fold with a 0.3-mM buffer of 2-(N-morpholino) ethanesulfonic acid (MES)₄, pH 6.1. Emergence was recorded under a stereomicroscope at 3-d intervals until no more germination occurred. When the germ tube was at least as long as it's the width of 90 to 130 mm, seeds were considered to have germinated and were removed. The seed viability test was carried out by slightly modified the procedures suggested by Khalaf (1991). Five milligram seeds were submerged for 5 min in 6% sodium hypochlorite solution to bleach the dark seed coat, then repeatedly washed in distilled water, dried in a flow of air at 40 °C, and imbibed on Whatman paper No. 1 in 6-cm-diam Petri dishes. In each dish, 3 ml of a 1% solution of 2,3,5triphenyltetrazolium chloride (TTC)5, prepared following International Seed Testing Association (1996) rules, was added, and the dish was incubated in the dark at 25 °C for 72 h. Red or pink seeds were considered viable, whereas those that were no color which were considered as dead [modified from Linke (1987)]. Seed viability was assessed using a binocular dissecting microscope.

Data recorded

Egyptian broomrape

The intensity of parasite infestation was evaluated by counting, seven times per season, between mid-November and May, the number of emerged Egyptian broomrape shoots per cucumber plant. Egyptian broomrape shoots from four cucumber plants for each plot were collected by hand in mid of April (both seasons) and weighed. In addition, at crop harvest, all cucumber roots of plants, grown in solarized soil were collected to record the presence of Egyptian broomrape haustoria and underground tubercles.

Cucumber crop

In both seasons, four aboveground plant samples were collected in mid-April, when the crop had reached its maximum growth rate, and fresh weight of leaves and stems was determined. Over the harvest period (from 106 to 213 DAP and from 113 to 220 DAP), the number and weight of ripe fruits harvested from 10 plants plot⁻¹ were recorded.

Statistical analysis

Both biological and production parameters were statistically analyzed through ANOVA where in the appropriate and mean separations were made by the following Fisher's protected LSD test. Regression analysis between Egyptian broomrape infestation and cucumber yield (and its components) were performed.

Mean temperature during the crop growth

Over the period of the crop cycle from October to early May, mean temperatures in the greenhouse maxima and minima (in °C) for the two seasons were 33.0 and 34.0 and 24.8 and 22.4, respectively.

Results

Soil temperature

Solarization substantially increased the soil temperature in both seasons and at both depths. The highest absolute maximum temperatures recorded at 5 cm depth in mulched soil were 54.4 and 56.2 °C in the first and second seasons, respectively, while it was 42.3 and 46.5 °C in bare soil.

The number of days when the maximum soil temperature equalled or exceeded 45, 50, 55and 60 °C at depths of 5 and 15 cm is shown in Table 1. Significantly, a temperature above 50°C at 5 cm was recorded for 31 (first season) and 51 (second season) d in solarized but for 7 (first season) and 18(second season) d in nonsolarized soil (Table 1). At 15 cm, a temperature above 50 °C was recorded for 2 (first season) and 18 (second season) d only in solarized soil (Table 1).

Egyptian broomrape control

In both years, Egyptian broomrape was completely controlled by soil solarization because the treatment did not allow the development and emergence of shoots, underground haustoria, or tubercles on the cucumber roots. In contrast, in nonsolarized soil, Egyptian broomrape shoots had emerged by 45 DAP (first season) and 56 DAP (second season), and by 86 DAP (95

DAP), there were as many as 43 (52) shoots per cucumber plant (Figure 1). At the end of the 2005 and 2006 cropping seasons, the number of Egyptian broomrape shoots per cucumber plant peaked at 133 (\pm 23) and 316 (\pm 21) with a fresh weight of 125 g (\pm 11) and 173 g (\pm 8), respectively. Germination of Egyptian broomrape seed sown at 5 and 15 cm was zero in solarized soil, whereas it reached 83 and 81%, respectively, in nonsolarized soil. The viability of buried seed at 5 and 15 cm was about 93% in nonsolarized soil but only 2% and 6% in solarized soil, respectively. No parasites other than Egyptian broomrape were found on the cucumber roots in either the solarized or the nonsolarized soil.

Table 1. Number of days when soil temperature was \geq 45, 50, 55, or 60 C during the solarization period at two soil depths.

	Treatment		5 cm				15 cm			
			≥ 50 C	≥55 C	≥60 C	≥45 C	≥50 C	≥55 C	≥60 C	
2005	Solarized soil	46	31	6	3	28	3	2	1	
	Nonsolarized soil	14	7	0	0	0	0	0	0	
2006	Solarized soil	53	51	31	12	47	18	1	2	
	Nonsolarized soil	22	18	1	0	0	2	0	0	

Cucumber plant growth and fruit yield

Plant growth and fruit yield were significantly higher in solarized soil than in nonsolarized soil. In mid Of April, when the cucumber plant reached its maximum size, soil solarization increased fresh leaf and stem weight per plant (Fig. 2). Yield accumulation was significantly affected by soil solarization (Fig. 3). In both seasons, fruit yield in solarized and nonsolarized plots was similar at the first harvest (106 DAP and 114 DAP), whereas starting with the second harvest, the yield rate of plants grown in solarized soil was significantly higher than the plants grown in nonsolarized soil (Fig. 3). At the beginning of April, the yield differences between solarized and nonsolarized plots had already reached 77% (3.9 vs. 2.2 kg plant⁻¹, first season) and 125% (3.6 vs. 1.6 kg plant2¹, second season). At the end of the harvest, plants grown in solarized soil produced 5.6 (6.8) kg plant⁻¹, compared with 2.4 (1.9) kg plant⁻¹ from plants grown in nonsolarized soil, equivalent to a yield increased of 133 and 258%, respectively. Plants grown in solarized soil produced 51.5 (55.8) fruits per plant, with an average fruit weight of 71.4 g (82.6 g), whereas plants grown in nonsolarized soil produced 26.5 (21.4) fruits per plant with an average weight of

38.3 g (57.3 g), respectively. Yield of Cucumber fruit (and its components) and infestation severity of Egyptian broomrape were negatively correlated (P < 0.001), as predicted.

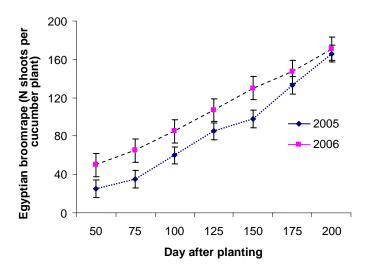


Fig. 1. Number of emerged Egyptian broomrape shoots during two growing seasons in nonsolarized soil. Note the failure of shoot emergence in solarized soil. Bars represent standard errors.

Discussion

Under such weather conditions [Iran (Hashtgerd)], soil solarization proved to be an excellent control method for total eradication of Egyptian broomrape infestation. In addition, solarization was able to consistently improve cucumber vield under greenhouse conditions, where as Egyptian broomrape is particularly destructive (Hodosy, 1981; Qasem, 1998; Qasem and Kasrawi, 1995). Without solarization treatment, Egyptian broomrape was consistently decreased fruit yield. As severe infestation increased (over the period of mid-December to May), the growth of cucumber plants was increasingly inhibited. The incidence of chlorosis increased, the photosynthetic rate was reduced (data not shown), and plants were visibly witted in the middle of the day, leads to yield collapse as early as mid-spring. Fruit yield and its components were negatively and significantly correlated with the number of Egyptian broomrape shoots per cucumber plant. Regression analysis associated with 80 and 606% of the variation in cucumber yield with the severity of the Egyptian broomrape attack for 2004 and 2006 cucumber crops, respectively. Soil solarization provided total control of Egyptian broomrape because shoot emergence from treated soil was completely inhibited,

and no haustoria or underground tubercles were found on the cucumber roots at the end of the crop cycle. The high soil temperature attained by mulching inhibited Egyptian broomrape germination killing about 95% of the buried seed and inducing secondary dormancy in the remaining 5%. This results are important because it indicates that the soil seedbank of Egyptian broomrape seed could be reduced after each solarization, and we predict that a number of consecutive solarizations was results in limiting the parasite seed number in the soil to a level where normal development of the cucumber crop is not compromised. The inhibition effects of soil solarization on broomrape seeds may be attributed to the lethal heat effect caused by the interaction of daily fluctuating elevated temperatures and accumulations of volatiles in the soil temperatures which functions as a means of sterilizing seed of Orobanche or disease pathogen in the soil (Kakesh and Foy, 1989). Field trials are planned to assess the degree of viability of Egyptian broomrape seeds buried naturally in the soil after various solarization treatments because we have previously demonstrated that the exposure of crenate broomrape seeds to high temperatures led to a significant progressive reduction in germination and a linear decrease in seed viability (Sahile et al., 2005 and Mauromicale et al., 2000).

Soil solarization improved cucumber plant growth and, consequently, fruit yield. The latter was 121 ± 7 and $232 \pm 18\%$ higher than in nonsolarized soil each season, respectively. Furthermore, yield levels were 85% (first season) and 81% (second season) above the average official greenhouse cucumber yield in Iran. These remarkable increases in cucumber yields by soil solarization are largely attributable to the absence of Egyptian broomrape infestation, but we cannot exclude additional beneficial effects generated by the solarization treatment, such as the control of soil-borne diseases, an increased release and uptake of macro and micronutrients, the release of plant growth regulators, the enhancement of mycorrhizal growth, and an increase in endogenous gibberellin supply (Chen and Katan 1980; Chen et al., 1991; Gru nzweig et al., 2000; Mauromicale et al., 2005). Given the high correlation between yield and Egyptian broomrape infestation, as well as the absence of soil-borne pathogens, and the optimal plant nutrition maintained over the crop cycle, the additional beneficial effects provided by soil solarization are probably minor compared with the negative effects of Egyptian broomrape populations. At present, Egyptian broomrape control in greenhouses in the Mediterranean basin and elsewhere is commonly controlled by regular preplant soil fumigation with methyl bromide; however, the use of this fumigant will be phased out by 2015 (2005 in the European Union). Therefore, Egyptian broomrape attack is currently a significant risk. Where legume cropping has been abandoned because of high Orobanche infection and low levels and stability of yield (Foti 1994; Mesa-Garcia and Garcia-Torres 1986), the adoption of soil solarization may be able to rescue these production areas. There are advantages to using solarization: specifically, it is a simple, nonchemical, nonhazardous method that avoids the use of any toxic materials, does not contaminate the site, and is, therefore, suited to organic farming or other low-input agricultural systems. As global environmental quality considerations grow in importance, along with an increasing human population, evolving concepts, such as soil solarization and other uses of solar energy in agriculture, will become more important (Stapleton 2000). Further research is necessary to determine the degree of Egyptian broomrape seedbank viability down the soil profile and its relationship with soil temperature reached during solarization to facilitate strategies to completely eradicate the parasite weed from the soil.

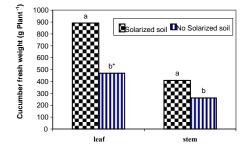


Fig. 2. Effect of soil solarization on the fresh weight of cucumber plant leaves and stem in mid-April averaged mean of the two seasons. (*, significant difference at $P \le 0.001$).

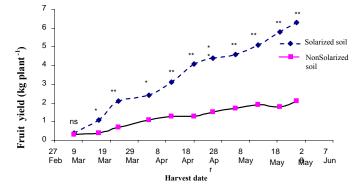


Fig. 3. The effect of soil solarization on cucumber yield under greenhouse conditions. Regression equation relating fruit yield accumulation at end harvest to infestation severity (number of emerged broomrape per cucumber plant) was y = 0.0711x - 2781, $r = 0.919^{**}$, R2 = 0.985 for the 2005 to 2006season; (*, **, and NS are significant differences at P ≤ 0.05 , 0.01, and not significant, respective.

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