



## SAFE CONTROL OF SOIL BORNE PATHOGENS OF BELL PEPPER PLANTS UNDER PLASTIC-HOUSE CONDITIONS

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### ABSTRACT

The experiments were conducted at the farm of Al-Alamia located at Nubaria- Egypt, in plastic houses (6m x 45m) where solanaceous crops have been grown as monoculture, and aimed to evaluate the soil solarization in combination with benefit microorganisms for controlling the pepper soil borne pathogens and weeds under plastic-house condition. Supplementation of organic matter in plastic-houses resulted in noticeable increase in fungal, bacterial and nematode counts. However, the population densities of total fungi, *Fusaria*, total bacteria, spore former bacteria, actinomycetes and nematodes after 15 and 30 days in the solarized plastic-houses were drastically reduced as compared to non solarized control soil. This reduction was gradually increased depended on the time of sampling (after 15 days of transparent polyethylene mulching or after 30 days). Soil solarization reduced sharply free nitrogen fixers, 30 days after treatment the elimination of the *Azotobacter* spp. and *Azospirillum* spp. from soil was recorded. However, the free nitrogen fixers were found to be recolonized after one month from transplanting the seedling pepper plants in non solarized and solarized soil, so artificial inoculation of pepper seedlings with strains of *Azotobacter* sp. and *Azospirillum* sp., were resulted great root colonization of plants than in non-solarized soil. Also, all annual weeds have been controlled with soil solarization which gave the best weed control treatment.

On the other hand, solarization had pronounced effect on seedling establishment occurred

in solarized plastic-houses. Increasing more than 30% in establishment of pepper seedling were recorded between the solarized and non-solarized plastic-houses which mulched with black sheets after three weeks of pepper transplanting. The percentage of *Phytophthora* spp. isolated from the infected pepper seedlings were very high compared with other fungi, it was 49% of total isolated fungi after one week of transplanting.

However, soil solarization increased pepper plant height and number of branches per plant as compared with non-solarized soil without black mulching or with black mulching. The pepper yield per plastic house up to 7 months increased with soil solarization by about 216 Kg, 18.3% over non solarized with black mulching, and 155 Kg, 12.4% over non solarized without mulching.

### INTRODUCTION

Bell pepper (*Capsicum annuum*) is among the most grown vegetables in greenhouses worldwide. It is a good source of antioxidants and nutrients, as well as bioactive compounds such as flavonoids, capsaicinoids, capsinoids, carotenoids and vitamins C, E, A and are also rich in natural colors and aromas (Jayaprakasha et al 2012). Also is considered one of the most important vegetable crops in Egypt. The average Egyptian annual consumption from pepper is about 5.4 kg/capita, which mean that the total Egyptian consumption is around 446000 tons/year (Anon, 2007). In addition pepper is one of the most important exportable crops in Egypt.

However, bell pepper is cultivated in the plastic-house conditions during the winter season that are favorable for many soil borne pathogens and weeds due to the high humidity. Soil borne pepper

diseases more commonly found in Egypt are damping-off and root rot caused by *Pythium* spp., *Rhizoctonia solani*, *Fusarium solani* (El-Mougy et al 2011); Fusarium wilt caused by *Fusarium oxysporum* f. sp. *capsici* (El-Mougy et al 2011 and Abdel-Monaim et al 2014); Phytophthora blight caused by *Phytophthora capsici* (Mosa et al 2002); Sclerotium wilt caused by *Sclerotium rolfsii* (El-Mougy et al 2011 and Abada and Ahmed, 2014); and root-knot nematodes caused by *Meloidogyne incognita* (Kesba, 2011). Such diseases are limiting factors for pepper growing in greenhouses. There are many methods, including chemical, cultural, and biological techniques, which have been developed for soil borne plant disease management. None of these methods are, however, effective enough to be used in all instances (Pullman et al 1981; Pinkas et al 1984; and Annesi and Motta, 1994).

An inexpensive and effective method, such as solarization (solar heating) of soil, is promising way to control soilborne pathogens including nematodes and weeds (Barbercheck and Broembsen, 1986; Elmore et al 1997; Gill et al 2009; and Zasada et al 2010). Application of fumigant, methyl bromide, is the most effective disinfestation technique of soils but it is a major ozone depleting substance. However, soil solarization is one of the most useful, non-chemicals and environmentally sound disinfestations methods (Elmore et al 1997). Soil solarization is accomplished by heating moist soil covered with a transparent plastic sheet, and it must be conducted before crops are established. Solarization has received much attention as a methyl bromide (MB) replacement, especially in some regions where MB has been extensively used (Zasada et al 2010). Over a 10-year period, there has been a reduction in MB of 85% (45,000 metric tons) used for pre-planting soil fumigation, meaning that a wide range of other chemicals and/or non-chemical technologies had been adopted for disease and weed control. Restrictions on the use of MB have stimulated new research, including (1) soil health and relationships between soil microbial diversity and crop growth and (2) new crop protection agents and production systems, including soil solarization, which reduces the need for pesticides in agriculture (Porter et al 2009). Solarization has been studied in over 60, both developed and developing, countries, mainly in the warm climate regions (Katan and Gamliel, 2009).

On the other hand, Plant growth-promoting rhizobacteria (PGPR) are free-living or root-

associated bacteria in the rhizosphere of many plant species that enhance plant growth, productivity and often elicit plant immunity against multiple plant pathogens (Ahmad & Kibret, 2014). Fluorescent pseudomonads are non-pathogenic rhizobacteria which suppress the soil-borne pathogens through rhizosphere colonization, antibiosis, iron chelation by siderophore production and induced systemic resistance (Vanitha & Ramjagathesh, 2014). Meantime *Azotobacter* and *Azospirillum* are the two most important non-symbiotic N-fixing bacteria in non-leguminous crops. Under appropriate conditions, *Azotobacter* and *Azospirillum* can enhance plant development and promote the yield of several agricultural important crops in different soils and climatic regions (Okon and Labendera-Gonzalez, 1994). These beneficial effects of *Azotobacter* and *Azospirillum* on plants are attributed mainly to an improvement in root development, an increase in the rate of water and mineral uptake by roots, displacement of fungi and plant pathogenic bacteria and, to a lesser extent, biological nitrogen fixation (Okon and Itzigshohn, 1995). Another important characteristic of *Azotobacter* association with crop improvement is secretion of ammonia in the rhizosphere in the presence of root exudates, which helps in modification of nutrient uptake by the plants (Narula and Gupta, 1986). The ability of *Azospirillum* to produce plant growth regulatory substances along with N<sub>2</sub> fixation stimulate plant growth and thereby productivity. The changes that occur in the plant roots help in transport of minerals and water (Sarig et al 1988). All these factors combined together produce positive effects on crop yield especially for vegetables and cereals. Unfortunately, soil solarization greatly reduced free nitrogen fixers (Abdel-Kader et al 1992).

The objective of this study was to evaluate the soil solarization in combination with benefit microorganisms i.e. *Pseudomonas fluorescens*, *Azotobacter* sp., *Azospirillum* sp. during production of pepper seedlings for controlling the serious soil borne diseases and weeds under plastic-house condition.

## MATERIALS AND METHODS

The experiments were conducted during growing season 2006 - 2007 at the farm of Al-Alamia located at Nubaria- Egypt, in plastic houses (6m x 45m) where solanaceous crops has been grown as monoculture.

The following treatments were applied on 6 pepper plastic-houses:

- 1- Two pepper plastic-houses with solar heating and amended with benefit microorganisms.
- 2- Two pepper plastic -houses without solar heating and without black mulching as control.
- 3- Two pepper plastic -houses without solar heating and with black mulching (which recommended to the growers).

#### Preparation of plastic-houses soil

All the plastic-houses were recovered by removing the plastic covers, tillage the soil, leveling and preparing the plant beds in the plastic-houses (3 plant beds and 2 sides).

Fifty kg of superphosphate, 1m<sup>3</sup> chicken manure and 2.5 m<sup>3</sup> cow manure were added to each pepper plastic-house, pre solar heating (solarization) in treatment with solar heating or just after the end of solarization period in treatments without solar heating. Manures were obtained from commercial sources and poultry farm. Both organic matter and superphosphate were mixed in soil with rototiller. The plastic-houses with solar heating were irrigated by dripping 3h /day for 9 days before solarization procedure. Solarization was carried out by covering the soil with transparent polyethylene 120 µm thick and 4 meter width and all of plastic-houses were exposed to full sunlight starting from June 29, till August 19.

#### Soil samples

Soil samples were taken before and after soil addition of organic matter as well as before and after soil mulching with transparent polyethylene (solarization) by using standard core auger. Five cores were collected per each plastic-house at depth ranges 5-10 cm. Samples from soil were taken periodically every 15 days. Samples from rhizosphere of pepper plant after transplanting also were taken.

#### Soil borne fungal diseases

##### A- Soil assays for total fungi and Fusaria

Samples were examined for total fungi and Fusarium spp. The soil dilution plate method reported by Mehrotra and Aggarwal (2003) and the plate count technique according to Allen (1961) were followed for fungal and fusaria counts using Martin's medium (Martin, 1950) and Nash &

Synder (1962) for total fungi and total fusaria, respectively in soil.

##### B- Seedling diseases assay

The percentage of seedling that appeared disease symptoms was recorded weekly for 3 weeks and calculated as  $A/B \times 100$  where A= number of diseased seedlings ; and B= number of total seedlings.

##### C- Isolation of the causal organisms

All seedlings which appeared disease symptoms were collected and transferred to laboratory of plant pathology, Ain-Shams University. Infected seedlings were washed carefully with tap water to remove the adjacent soil particles. The washed basal parts of seedlings were cut into small pieces by a sterilized knife. Specimens of suitable size were surface sterilized by immersing the root pieces in sodium hypochloride (1% active chlorine) for 2-3 minutes (Waller, 1981), and then washed by sterilized distilled water. The surface sterilized plant materials were dried between two sterilized filter papers and placed in Petri-dishes containing water agar medium, and incubated at 23°C for 3-6 days. The growing fungi were examined microscopically and were either hyphal tipped or single spore carefully transferred to slopes of PDA medium, (Keitt, 1915). Pure cultures of each isolate were maintained on PDA slants and kept at 4±1°C for further experiments. Identification studies were made by studying the cultural and microscopic characteristics of each isolate of fungi isolated according to Barron, 1968; Sneh et al 1991; Barnett & hunter, 1998; Alexopoulos, 1996 and Webster, 1980.

##### Procedures for counting soil microorganisms

Samples were examined for total bacteria, spore former bacteria, actinomycetes, and two nitrogen fixeres (*Azotobacter* & *Azospirillum*). The soil dilution plate method and the plate count technique according to Allen (1961) were followed for bacterial, Actinomycetes and spore former counts. Glucose soil extract agar medium (Bunt and Rovira, 1955) was used for total bacteria and spore former bacteria. *Azotobacter* spp. counts were determined using modified Ashby's medium (Abd-El-Malek & Ishak 1968); modified Doberener's medium (Tyler et al., 1979) was used for counting *Asospirillum* spp. However, starch medium were used for actinomycetes counts (Allen 1961).

### Extraction of nematodes from soil and identification

Periodically occurred sufficient sub-samples of soil were collected and thoroughly mixed together to form a composite soil sample of approximately 250 cc soil. Samples were kept separately in polyethylene bags and prepared in the same day for nematode extraction by the Seinhorst method (Seinhorst, 1962) in nematode assay laboratory at Plant Protection Dept., Ain Shams University. The extracted nematodes were counted and identified microscopically according to (Zuckerman et al 1971 and Fortuner, 1988).

### Preparation of seedlings

**A- Potting medium:** consisted of peat moss, vermiculite (1:1 vol.), plus basic fertilizers containing (250 gr.) ammonium nitrate, (150 gr.) potassium sulfate, (24 gr.) magnesium sulfate, (400 gr.) superphosphate, seventy five cc foliar fertilizer containing micronutrients, and 4 kg calcium carbonate per 50 kg peat moss, for potting medium and watered to saturation. The potting medium was mixed then kept in loosely tied plastic bags for two days. On the other hand, the same medium plus 50 gr. Topsin-M / 50 Kg peat moss was in treatments without solar heating.

**B- Containers of transplants:** new foam punnets (seedling trays) each consisted of eighty four inverted pyramid shape cells 4 × 4 cm at the top 6 cm deep, and 77 cm<sup>3</sup> capacity were filled with potting medium and used for transplant production.

**C- Preparation of inocula:** the bacterium *Pseudomonas fluorescens*, previously isolated by the authors (Mosa et al 1997), as well as *Azotobacter* sp., and *Azospirillum* sp. which previously isolated from plastic-houses under study were used for bacterial inocula. The inocula were prepared immediately before inoculation. Modified Ashby's medium (Abd-El-Malek & Ishak 1968), modified Doberener's medium (Tyler, 1979), and nutrient yeast-dextrose broth (NYDB) medium (per litre: nutrient broth 8 g, yeast extract 5 g and dextrose 10 g) were used for production of inocula of *Azotobacter* sp., *Azospirillum* sp. and *P. fluorescens*, respectively in 500 cm<sup>3</sup> flasks. The flasks were placed on a rotary shaker at 120 rpm for 66 hrs at 24±1°C. At the time of seeding, Two ml 2x10<sup>8</sup> (cfu) of the *P. fluorescens* medium and 7 days after

seeding, one ml 2x10<sup>8</sup> (cfu) of each the *Azotobacter* sp. and *Azospirillum* sp. media together were pipetted onto the surface of the potting medium in each cell of punnets.

**D- Seeding:** punnets were seeded with pepper cultivar 'Cloves', one seed per cell on July 25. Overhead liquid fertilization was applied after twenty days and repeated on a ten days schedule.

### Transplanting

The pepper seedlings were transplanting on September 9, in six plastic-houses. Two plastic-houses treated by solar heating were transplanted with pepper seedlings treated by bioagent and nitrogen fixers (*Azotobacter* sp., and *Azospirillum* sp.) bacteria. Two plastic -house without solar heating were transplanted with pepper seedlings which were not treated by bioagent and nitrogen fixers without using black mulching. Two plastic-houses without solar heating were transplanted with pepper seedlings which were not treated by bioagent and nitrogen fixers with using black polyethylene mulching. The last four plastic-houses were treated by the fungicide Topsin-M 1 gr/L twice on the date of transplanting and 15 days later. Re-transplanting was done twice, 15 and 25 days from transplanting.

### Weeds survey

At the end of solarization period, soil samples were collected from both solarized and non-solarized plastic houses at 0-5 cm depth. The soil samples transferred to 10 x 10 x 40 cm container for weed germination test and weed counts. Moreover, weed groups present in each plastic house and their dry weight were recorded from a quadrat 50 cm x 50 cm thrown four times randomly after 2 and 4 weeks from pepper transplanting in plastic houses.

### Morphological growth

Plant height and number of branches per plant were recorded every four weeks, 10 plants from each replicate as a sample, 40 plants from each treatment to study the effect of solarization with benefit microorganisms on plant growth and yield.

### Statistical analysis

Three treatments each consisting of four replicates were used in this experiment. The replicate was one half of one plastic-house. The obtained data were subjected to computer statistical software (ASSISTAT) originated by **Silva & Azevedo (2009)** and Randomized blocks design (RBD) was conducted. Data analyzed using analysis of variance (ANOVA), and mean values were compared using Duncan's multiple range test at a significance level of  $P \leq 0.05$ .

**RESULTS AND DISCUSSION**

**Effect of soil solarization on the total fungi**

The numbers of total fungi were determined in two plastic-houses with solar heating and four plastic-houses without solar heating in different periods i.e. pre & post solarization and pre & post amended with organic matter. Results in **Table (1)** indicated that the numbers of total fungi were decreased sharply after 30 days of mulching with transparent polyethylene (solarisation). Meantime, at the end of the solarization period (50 days of mulching), the total fungi increasing in untreated plastic-houses because of the addition of organic matter to untreated plastic-houses was at this time. Transplanting the seedlings of pepper in plastic-houses caused increasing in the total fungi

counted in the solarized soil. However, the total fungi counts were decreased sharply in the non-solarized plastic-houses, after 4 weeks of pepper transplanting, result from using of fungicides (Top-sin-M) as soil treatments in those plastic-houses at this period.

However, the total count of fungi increased in the soil of the non-solarized plastic-houses which covered with black mulch than those which not covered with the black mulch, these results may be correlated with the humidity difference in the soil between mulched and non-mulched soil.

In this respect, solarization creates a partial biological vacuum in the soil. Although heat tolerance varies among organisms, generally only minutes are required at temperatures above 45°C to reach LD<sub>90</sub> levels (**Stapleton, 1991**). However, populations of mesophilic organisms decline at faster rates during solarization. For these organisms, accumulation of heat effects above 37°C over time is lethal (**DeVay, 1991**). The inability of fungi to tolerate high temperatures is related to an upper limit in the degree of fluidity of membranes, beyond which breakdown of membrane function may be associated with membrane instability. Additional causes for the thermal decline of microorganisms at high temperatures involve the sustained inactivation of respiratory enzymes (**Brock, 1978**). These are direct affects of high soil temperatures and account for a major share of the reduction in populations of soilborne micro-organisms.

**Table 1.** Population densities of total fungi<sup>1</sup> in the soil of pepper plastic houses (10<sup>4</sup> cfu/g dry weight of soil) during 2006 growing season

Solarized		Non- solarized		
Sampling time	Solarized	Sampling time	without black mulching	with black mulching
Before organic matter June 21,	0.18 e	Before organic matter June 21	-	0.13 e
7 days after organic matter June 29,	43.0 a	Before organic matter July 15	-	0.14 e
15 days of solarisation July 15	0.06 e	Before organic matter July 15	-	0.70 e
30 days of solarisation July 30	0.03 e	Before organic matter July 30	-	0.59 e
50 days of solarization Augst 19,	0.03 e	7 days after organic matter Augst 27,	-	25.30 b
4 weeks of transplanting October 9,	3.75d	4 weeks of transplanting October 9,	7.5 c	8.6 c

1- Total fungi counts was done using Martin's medium

2- Means followed by the same letter are not significantly different according to Duncan's multiple range test, (p = 0.05).

### Monitoring soil populations of *Fusarium* spp.

At the start of experiment, sandy soil in all under investigation plastic-houses contained relatively low counts of *Fusarium* being in the range 0.23-0.70 x 10<sup>3</sup> cfu /g dw soil which is mainly due to lack of organic matter. Supplementation of organic manure in plastic-houses resulted in noticeable increase in fungal counts over the untreated soil. Results in **Table (2)** showed that *Fusarium* spp. populations were drastically reduced at solarized treatment after 15 and 30 days as compared to untreated plastic-houses, the solarization reduced fusaria populations sharply till zero cfu/g dry soil after 15 days of solarization until the end of solarized period (50 days). The amount of Fusaria populations appeared to increase after 4 weeks of pepper transplanting in plastic houses but, the population of fusaria in solarized soil still lower than the non-solarized soil even though, using of fungicides Topsin-M either in plastic-houses which mulched with black sheets or not. **DeVay (1991)** stated that solarization is especially effective, in regions with high solar radiation and where daily temperatures reach 32°C or higher and that maximum soil temperatures in the first 5 cm are commonly between 42°C and 55°C. However, there

are reports of good pathogen control with soil solarization (**Davis and Sorensen, 1986;** and **Swaminathan et al 1999**).

### Establishment of pepper seedling after transplanting

Solarization had pronounced effect on seedling establishment occurred in solarized plastic-houses. Increasing more than 15% in establishment of pepper seedling were recorded between the solarized and non-solarized plastic-houses which mulched with black sheets after one week of pepper transplanting. However, the reduction of establishment of pepper seedling in the non-solarized without black mulching was recorded 9.43% (**Table 3**). The percent of establishment reduction of pepper seedlings appeared also after 2 and 3 weeks of transplanting in non-solarized plastic-houses which mulched with black sheets and achieved 10.59 and 5.77%, however the reduction reached to 0.15% after 3 weeks of transplanting in solarized one. Also, numbers of diseased plants significantly were increased with black polyethylene mulching. In addition, the seedling of pepper seedlings in solarized plastic-houses had more uniform growth rate than seedling in the non-solarized plastic-houses.

**Table 2.** Population densities of total *Fusarium* spp.<sup>1</sup> in the soil of pepper plastic houses (10<sup>3</sup> cfu/g dry weight of soil) during 2006 growing season

Solarized		Non- solarized		
Sampling time	Solarized	Sampling time	without black mulching	with black mulching
Before organic matter June 21,	0.23 d	Before organic matter June 21	-	0.70 d
7 days after organic matter June 29,	7.00 b	Before organic matter June 29,	-	0.45 d
15 days of solarisation July 15	0.00 d	Before organic matter July 15	-	0.33 d
30 days of solarisation July 30	0.00 d	Before organic matter July 30	-	0.20 d
50 days of solarization August 19,	0.00 d	7 days after organic matter August 27,	-	11.10 a
4 weeks of transplanting October 9,	1.00 d	4 weeks of transplanting October 9,	6.00 c	7.50 b

1- Total fusaria counts was done using Nash & Synder's medium

2- Means followed by the same letter are not significantly different according to Duncan's multiple range test, (p = 0.05).

**Table 3.** Percentage of reduction in seedling establishment of pepper in plastic houses in different treatment

Samples time	Solarized	Non- solarized	
		without black mulching	with black mulching
1 weeks of transplanting	3.43 de	9.43 b	18.93 a
2 weeks of transplanting	0.80 ef	4.02 cd	10.59 b
3 weeks of transplanting	0.15 f	2.12 e	5.77 c
<b>Total</b>	<b>4.38</b>	<b>15.57</b>	<b>35.29</b>

1- Means followed by the same letter are not significantly different according to Duncan's multiple range test, ( $p = 0.05$ ).

However, the percentages of the fungi associated with the diseased seedlings of pepper plants in plastic-houses were calculated (**Table 4**). The percentage of *Phytophthora* spp. isolated from the pepper seedlings were very high compared with other fungi, it was 49% of total fungi isolated from pepper seedlings followed by *Alternaria* spp. which reached 22.3% of total isolated fungi after one week of transplanting. The high percentages of the isolation of *Phytophthora* spp. and *Alternaria* spp. were related to using Topsin-M as soil drench fungicide in untreated plastic-houses of pepper. Many reports indicated that thiophanate-methyl the active ingredient of Topsin-M is not effective against *Phytophthora* spp. or against *Alternaria* spp. (**Ishii, 2003** and **Chase, 1992**).

#### Effect of soil solarization on bacterial microorganism

Sandy soil in the six plastic-houses showed little variation in total bacterial counts, spore former, and actinomycetes. Supplementing sandy soil in two plastic-houses with organic matter (cow manure, poultry manure) and irrigation resulted in obvious increase in total bacteria, spore former and actinomycetes over untreated plastic-houses, which may be due to the presence of easily decomposable organic matter and of high nitrogen content (**Table 5**). Organic matter in soils may affect plant growth through the following mechanisms: a) Serving as a source of mineral N. b) Serving as a C, N, and energy source to beneficial soil microorganisms affecting plant growth. c) The soluble fraction of soil organic matter (fulvic acid and humic acid) may serve as a complexing agent and a carrier for microelements to the roots. d) The soluble fraction may have direct effects on plant growth when taken up (**Chen et al 1991**). In fact, soil microorganisms required approximately the same nutrients as do crop plants, therefore, applica-

tion of organic manure as a fertilizer, which are major factor in maintaining soil fertility. Results also showed that the population densities after 15 and 30 days in the solarized plastic-houses were drastically reduced as compared to untreated control soil. This reduction was gradually increased depended on the time of samples (after 15 days of mulching or after 30 days of mulching). At the end of the solarization period (57 days of mulching) the population densities of total bacteria increasing in untreated plastic-houses ( $380.3 \times 10^6$  cfu/g dry soil) compared with  $0.03 \times 10^6$  cfu/g dry soil in solarized plastic houses resulting from the addition of organic matter to untreated plastic-houses before this time.

Transplanting the seedling pepper in plastic-houses caused increasing in the population densities of total bacteria especially in solarized soil counted in the rhizosphere. However, population densities of total bacteria were decreased in the non-solarized plastic-houses, after 4 weeks of pepper transplanting, may be resulting from using of fungicides as soil treatments in these plastic-houses at these periods (**Table 5**).

However, the initial counts of *Azotobacter* spp. and *Azospirillum* spp in the sandy soil before addition of organic manure were found in the range of  $1.8 - 2 \times 10^3$  and  $1.2 - 1.1 \times 10^4$  respectively (**Table 5**). Addition of organic manure stimulated free nitrogen fixers, but the soil solarization reduced sharply free nitrogen fixers. After 15 and 30 days of treatment the elimination of the *Azotobacter* spp. and *Azospirillum* spp. from soil was completely. After one month from transplanting the seedling pepper plants in untreated and solarized soil, the free nitrogen fixers were found to be recolonized. So artificial inoculation of pepper seedlings, with strains of *Azotobacter* sp. and *Azospirillum* sp., were resulted better root colonization of plants than in non-solarized soil (**Table 5**).

**Table 4.** Percentage of the fungi isolated from the infected seedlings of pepper plants in plastic-houses after different weeks of transplanting

Isolated fungi	After one week			After two weeks			After three weeks		
	A	B	C	A	B	C	A	B	C
<i>Fusarium</i> spp.	21.3	0.0	0.0	18.2	43.6	37.3	0.0	0.0	21.5
<i>Phytophthora</i> spp.	21.3	71.3	54.4	45.4	12.7	49.7	0.0	65.5	57.0
<i>Rhizoctonia</i> spp.	0.0	28.7	0.0	18.2	10.9	0.0	0.0	0.0	0.0
<i>Alternaria</i> spp.	21.3	0.0	45.6	18.2	32.7	13.1	50.0	34.5	21.5
Other	36.1	0.0	0.0	0.0	0.0	0.0	50.0	0.0	0.0
Total number of infected plants in each treatment	47	129	259	11	55	145	2	29	79

A: solarized

B: Non- solarized without black mulching

C: Non- solarized with black mulching

**Table 5.** Dynamics of total bacteria, actinomycetes, spore former bacteria, *Azotobacter* spp. and *Azospirillum* spp. populations in pepper plastic-houses

Sampling time	Treatment	Bacteria	Actinomy.	S. former	Azotobac.	Azospir.
		$10^6$ CFU/g	$10^5$ CFU/g	$10^5$ CFU/g	$10^3$ CFU/g	$10^4$ CFU/g
Before organic matter June 21,	A	11.20	8.7	3.0	1.8	1.20
7 days after organic matter June 29,	A	450.00	18.0	12.9	14.7	12.40
15 days of solarisation July 15	A	0.36	1.2	0.65	0.9	0.14
30 days of solarisation July 30	A	0.03	0.87	0.04	0.0	0.00
50 days of solarization Augst 19,	A	0.03	0.96	0.05	0.0	0.00
4 weeks of transplanting October 9,	A	136.00	7.5	7.5	650	92.10
Before organic matter June 21	B	-	-	-	-	-
Before organic matter June 29,	C	6.50	4.5	4.8	2.0	1.10
Before organic matter July 15	B	-	-	-	-	-
Before organic matter July 30	C	4.50	2.2	1.5	1.5	0.37
Before organic matter July 15	B	-	-	-	-	-
Before organic matter July 30	C	1.45	3.1	1.3	1.1	0.21
Before organic matter July 30	B	-	-	-	-	-
Before organic matter July 30	C	1.20	2.2	0.3	1.0	0.03
7 days after organic matter Augst 27,	B	-	-	-	-	-
7 days after organic matter Augst 27,	C	380.30	14.5	13.8	12.9	6.60
4 weeks of transplanting October, 9	B	63.00	8.4	6.4	3.8	2.40
4 weeks of transplanting October, 9	C	57.00	9.3	6.8	3.2	2.80

A: solarized

B: Non- solarized without black mulching

C: Non- solarized with black mulching

**Effect of soil solarization on nematodes population's dynamics**

Results presented in **Table (6)** indicate the presence of free living nematodes, such as *Rhabditis* spp. and *Dorylaimus* spp. are the dominant genera in the soil particularly after amended with organic matter. Although plant parasitic nematodes (mainly *Melodogynae* spp.) are serious pests on vegetables, their presence were low. Apparently, maximizing of soil temperature by solarization process was effective in maintaining the tested nematodes in the lowest level. However, under non solarized control soil free living nematodes responded numerically to enrichment of mixed soil with organic matter and plant parasitic nematodes were recorded. Soil solarization is a technique, which has shown promise for the control of several soil-borne pathogens and weeds in warm areas (**Katan, 1987**). The use of soil solarization for control of nematodes has received increasing attention (**Greco et al 1985; Barbercheck and Von**

**Broembsen, 1986; Nico et al 2003 and McSorley et al 2009**). The efficacy of soil solarization is based on the sensitivity of nematodes to relatively high temperatures. **Endo (1962)** demonstrated that the time required killing 100 percent juveniles within cysts of *Heterodera glycine* is temperature dependent. He found that 8 minutes, and 8 hours were required to inhibit egg hatch of the nematode at 52°C, and 44°C, respectively. Similar lethal temperatures (5 min exposure at 55°C) are reported for *Globodera rostochiensis* (**Mai and Lautz, 1953**). Notwithstanding that under clear plastic mulch, temperatures higher than 50°C can be reached only in the top 5 cm of the soil, temperatures of 40-50°C have been reported up to 10-15 cm depth in hot seasons in several countries. Moreover, temperatures of 36-40°C can be reached at 20-30 cm depth in warm areas. Such temperatures, if prolonged, can be lethal to nematodes or at least may reduce their infectivity because of energy reserve depletion (**Lamberti and Greco, 1991**).

**Table 6.** Nematodes population's dynamics in pepper plastic-houses

Sampling time	Treatment	Parasitic nematodes 100 cm <sup>3</sup> soil	Free living nematodes 100 cm <sup>3</sup> soil
Before organic matter June 21,	A	3	45
7 days after organic matter June 29,	A	23	143
15 days of solarisation July 15	A	0	0
30 days of solarisation July 30	A	0	0
50 days of solarization August 19,	A	0	0
4 weeks of transplanting October 9,	A	0	4
Before organic matter June 21	B	-	-
Before organic matter June 29,	C	2	39
Before organic matter July 15	B	-	-
Before organic matter July 30	C	2	28
Before organic matter July 15	B	-	-
Before organic matter July 30	C	1	21
Before organic matter July 30	B	-	-
Before organic matter July 30	C	1	9
7 days after organic matter August 27,	B	-	-
7 days after organic matter August 27,	C	19	120
4 weeks of transplanting October, 9	B	5	23
4 weeks of transplanting October, 9	C	6	19

A: solarized

B: Non- solarized without black mulching

C: Non- solarized with black mulching

### Effect of soil solarization on pepper weeds

Weeds of every a quadrant sample separated and classified into two groups: annual broad-leaved weeds and annual grass weeds. Because no perennial weeds obtained from a quadrat samples, the dry weight of weeds per unit area could be used as a reliable index to distribution. The results of the effects of soil solarization treatment on weed control in pepper plastic houses are presented in **Table (7)**. All annual weeds have been controlled with soil solarization which gave the best weed control treatment. In this respect, weeds are unwanted plants playing a very important role in different eco-systems and many of them cause enormous direct and indirect losses. Weeds are considered to be a potential pest causing more than 45% loss in yields of field crops,

when compared to 25% due to diseases (**Gnanaivel and Natarajan, 2014**). Solarization can increase soil temperature to levels that kill many weed seeds and seedlings. It leaves no toxic residues and can be easily used on a small or large scale. The basic phenomenon helping weed control upon soil solarization is build up of lethally high temperatures in top soil where most of the dormant and viable weed seeds are present. The possible mechanisms of weed control by soil solarization are breaking dormancy of weed seeds and solar scorching of emerged weeds and direct killing of weed seeds by heat. Soil solarization increases soil temperatures by 8 to 12°C over the corresponding non-mulched soil (**Hosmani and Meti, 1993**). Soil solarization with 0.05 mm thickness for 40 days recorded significantly higher pod yield of ground nut and least weed seed reserves in the top 5 cm soil (**Sundari and Sureshkumar, 2008**).

**Table 7.** Effect of soil solarization on weed control in pepper plastic houses as compared with Non- solarized soil without black mulching or with black mulching, 2 and 4 weeks from transplanting

Treatments	Annual grasses/m <sup>2</sup>				Annual broad-leaves weeds/m <sup>2</sup>			
	2 Weeks		4 Weeks		2 Weeks		4 Weeks	
	Number	Dry Weight g.	Number	Dry Weight g.	Number	Dry Weight g.	Number	Dry Weight g.
Solarized soil	0 c	0.0 c	0 c	0.0 c	0 c	0.0 c	0 c	0.0 c
Non-solarized without black mulching	91 a	48.2 a	264 a	156.0 a	273 a	78.4 a	209 a	126.0 a
Non- solarized soil with black mulching	39 b	3.3 b	21 b	2.8 b	48 b	4.0 b	27 b	2.5 b

\*Means in each column followed by the same letter are not significantly different according to Duncan's multiple range test, ( $p = 0.05$ ).

### Effect of soil solarization on Weed germination

The results of the effect of soil solarization on weed seed germination in plastic –houses soil surface (0 - 5 cm depth) are presented in **Photo (1)**, which indicate that seeds of all weed species did not germinated in solarized soil on the contrary with non solarized soil.



**Photo 1.** Weed germination test, non-solarized soil at right while solarized soil at left.

**Effect of soil solarization on morphological growth and yield of pepper plants**

The effects soil solarization of plastic houses on number of branches per pepper plant up to 8 weeks, plant height up to 16 weeks, and on the plastic house yield up to 7 months were recorded in **Tables (8, 9 and 10)**. The Results showed that soil solarization increased weekly pepper plant height and number of branching per plant as compared with Non-solarized soil without black mulching or with black mulching. The pepper yield per plastic house up to 7 month increased with soil solarization by about (216 Kg) and (155 Kg) as compared with Non-solarized soil with mulching and Non-solarized soil without mulching (control) plastic houses respectively. These results may be due to the faster of growth and increased size and appearance for crop plants. This phenomenon can be attributed, in part, to pathogen and weed control. However, a partial explanation of this phenomenon may be found in a combination of mechanisms. First, because major pathogens and pests are controlled by solarization, it is likely that minor or unknown pathogens and pests are also controlled. The unexpected beneficial effect on plant growth in the absence of a target pest was verified

and is being recognized as an important side effect with all disinfestation methods, including solarization (**Chen & Katan, 1980; Stapleton & DeVay, 1982 and 1983; Luis et al 2012**). Increased growth response (IGR) is a term commonly used to describe this phenomenon. Different mechanisms have been suggested to explain the stimulation of plant growth in solarized soils such as (**Avisar et al 1986**): (a) elimination of unknown minor pathogens; (b) chemical changes in the soil, concentrations of nitrate-nitrogen (NO<sub>3</sub>) and ammonium (NH<sub>4</sub>) have been increased up to six times compared with non solarized soils (**Stapleton and Devay, 1986**); (c) inactivation of phytotoxic substances in the soil (**Katan, 1981**); (d) stimulation of beneficial microorganisms such as mycorrhizal fungi, *Thichoderma*, *Aspergillus*, *actinomycetes*, *Bacillus* and *Pseudomonas*, which recolonize the soil quickly once normal temperatures are restored. The increases in NH<sub>4</sub> N and NO<sub>3</sub> N in solarized soils are likely due to the release of labile NH<sub>4</sub> N from soil microbial populations and subsequent nitrification (**Law et al 2008**). **Ghini et al (2003)** documented that in solarized plots there was a significant increase in the concentration of NH<sub>4</sub> N, Mn, NO<sub>3</sub>- N, Mg and K, however the concentration of Cu, Fe, Zn, H and Al decreased.

**Table 8.** Effect of soil solarization on number of branches of pepper plants after 4 and 8 weeks from transplanting

Samples time	Solarized	Non- solarized	
		without black mulching	with black mulching
4 weeks of transplanting October, 9	5.5 b	3 c	2.6 c
8 weeks of transplanting November, 6	5.7 a	5.5 b	5.3 b

1- Means followed by the same letter are not significantly different according to Duncan's multiple range test, (p = 0.05).

**Table 9.** Effect of soil solarization on plant height of pepper plants after 4,8,12, and 16 weeks from transplanting

Treatments	Samples time			
	After 4 weeks of transplanting October, 9	After 8 weeks of transplanting November, 6	12 weeks of transplanting December, 4	16 weeks of transplanting January, 6
Solarized soil	31.7 a	74.2 a	86.1 a	88.4 a
Non-solarized without black mulching	23.7 c	68.6 ab	78.1 b	82.3 b
Non- solarized soil with black mulching	27.7 b	65.0 bc	76.8 b	78.5 b

\*Means in each column followed by the same letter are not significantly different according to Duncan's multiple range test, (p = 0.05).

**Table 10.** Effect of soil solarization on plastic houses yield (kg) of pepper plants during seven months from transplanting

Samples time	Treatments		
	Solarized soil	Non-solarized without black mulching	Non- solarized soil with black mulching
1 <sup>st</sup> . Month October, 10	0	0	0
2 <sup>nd</sup> . Month November, 10	55	45	26
3 <sup>rd</sup> . Month December, 10	392	383	346
4 <sup>th</sup> . Month January, 10	290	256	241
5 <sup>th</sup> . Month February, 10	225	180	185
6 <sup>th</sup> . Month March, 10	226	192	195
7 <sup>th</sup> . Month April, 10	208	185	187
Total	1396	1241	1180

In conclusion this research confirms the potential of soil solarisation amended with beneficial microorganisms to control soil borne pathogens and weeds. However, other work is needed in the direction of economic analysis to estimate the cost of chemicals, black polyethylene, retransplanting of more than 30% of pepper seedlings in non solarised soil with black mulch (recommended to the growers) compared to the cost of solarisation process with transparent polyethylene and environmental benefits.

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