The Bioscan 8(4): 1177-1182, 2013
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

EFFECT OF SOIL - SOLARIZATION INTEGRATED WITH ORGANIC AMENDMENTS AND BIOAGENTS ON POPULATION DYNAMICS OF SOIL MICROBIAL POPULATIONS

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KEYWORDS
Soil-solarization
Mulching
Organic amendments
Bioagents
c.f.u Fungi
Bacteria
Actinomycetes
Trichoderma spp.
Pseudomonas fluorescens

Received on :
09.04.2013

Accepted on :
22.10.2013

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Abstract
Soil-solarization, a hydrothermal process, is very simple, cheap and non hazardous technique, to cover airtight the moist soil with polyethylene mulches/sheeting during the period of intense solar radiations for the particular duration results in increases in the soil temperature. In the present investigations, plots for raising nurseries of vegetable crops were mulched with thin transparent polyethylene sheets for 8 weeks. During Solarization, the temperature on an average was 10 to 12°C higher than the unmulched soil. The plot (size-1m²) before mulching with transparent polythene sheets amended with organic amendments farm yard manure (2 kg/plot), poultry manure (2 kg/plot)) and bioagents *Trichoderma harzianum* (6 gm/plot), *Pseudomonas fluorescens* (6 gm/plot)) further increase soil's suppressivity potential. The microbial population existing in soil was modified due to the hydrothermal process. Total populations of fungi, bacteria, actinomycetes, *Trichoderma* spp. except *Pseudomonas fluorescens* decreased drastically and significantly due to the solarization for 8 weeks. The microbial population (c.f.u) recorded in solarized plots just after soil-solarization of 8 weeks were as follows: fungi (13 to 27 $\times$ 10^{-3}/g soil), bacteria (40 to 61.23 $\times$ 10^{-6}/g soil), actinomycetes (3.66 to 8.33 $\times$ 10^{-5}/g soil) , *Trichoderma* spp. (15.44 to 28.22 $\times$ 10^{-3}/g soil) and *Pseudomonas fluorescens* (52 to 68 $\times$ 10^{-6}/g soil). In non-solarized plots, the population count (c.f.u) of fungi (31 to 56 $\times$ 10^{-3}/g soil), bacteria (70 to 99.56 $\times$ 10^{-6}/g soil), actinomycetes (6.83 to 13.33 $\times$ 10^{-5}/g soil) and *Trichoderma* spp. (24.44 to 41.44 $\times$ 10^{-3}/g soil) and *Pseudomonas fluorescens* (31.66 to 40.33 $\times$ 10^{-6}/g soil). The microbiological changes of naturally existing populations of fungi, bacteria, actinomycetes, *Trichoderma* spp. were decreased and of *P. fluorescens* were increased by solarization carried out for 8 weeks. However, after 30 days i.e. after raising a nursery crop, the estimated population showed significant recovery except the population of *P. fluorescens,* changed only marginally.

INTRODUCTION

Soil solarization is the covering of soil with polythene sheets produces green house effect resulted in raising soil temperature commonly to 35-36°C, during hot months of the years, as polyethylene reduces heat convection and water evaporation from the soil to the atmosphere (Silverstein,1976). Solar heating involves the use of heat as a lethal agent for pest control through the use of traps or capturing solar energy by means of transparent soil mulches to increase temperature to the extent lethal to the soil-borne plant pathogens (Katan,1981). Soil-solarization a passive but complex phenomena, comprises of physical, chemical and biological components involving the physicochemical and biological changes used to occurred during and after solarization which contribute to the biocidal effect (Stapleton *et al*., 1985; Ristaino *et al*., 1996). Solarization also resulted in a significant reduction in microbial populations in a combination of all the three techniques, viz physical control (soil solarization), cultural method (organic amendments) and biological control (*Trichoderma* spp.) (Joshi *et al*., 2009).

Soil solarization decreased the microbial populations of fungi, bacteria and actinomycetes (Sharma and Sharma, 2002 ; Joshi *et al*., 2009). Microbial load exhibited a significant decrease in solarized plots (Sharma and Razdan,2011) but the populations of *fluorescent pseudomonads* bacteria in solarized plot were increased significantly (Sastry and Chattopadhyay, 1999).

Solarization and organic residues amendments have been evaluated as disease control strategies with good perspectives for application in the integrated management of plant diseases (Collina, 2005; Baptista *et al*., 2006). Supplementation of the soil with organic matter prior to solarization has been proposed as a alternative management option. Combinations of the amendments with soil solarization were more effective than the amendments or soil solarization alone (Okaya *et al*., 2007). The population of fungi, bacteria and actinomycetes in soil, reduction was considerably higher in solarized soils amended with organic manures as compared to non-amended solarized soils (Joshi *et al*., 2009). Organic supplementation increased the maximum soil temperature, achieved through solarization by 3.9 to 4.7º C or 3.9 to 10.5 º C (Raj,2004; Mauromicale *et al*., 2010). Moreover, the organic amendments exert a protective role keeping soil microbial biomass and enzymatic activities protected from the detrimental effect of heating. (Scopa and Dumontet, 2007).

The concept of solarization is based on the fact that most plant pathogens and pests are mesophilic and get killed directly or indirectly. The higher temperature is unsuitable for most of the plant pathogens results decreased in their population (Shukla and Dwivedi, 2011). Thus, Soil solarization, a promising technique, is a pre-planting treatment not based on chemicals, used in hot climates to control soil-borne pathogens (Bonanomi *et al*., 2008; Ijoyah and Koutatouka, 2009; Mauromicale *et al*, 2010). The objective of our study is to study the effect of soil-solarization integrated with organic amendments and bioagents on microbial populations (fungi , bacteria, *Trichoderma spp.*, *pseudomonas fluorescens* and actinomycetes).

MATERIALS AND METHODS

Soil samples were collected from each replication plots at 5 cm depth just after mulching the plot with transparent polyethylene sheets (for 8 weeks) and after 30 days of raising the vegetable crops, on solarized and non-solarized plot from rhizosphere and non-rhizosphere zone. Isolations from soil to assess population dynamics were done using dilution plate technique (Walksman and Starkey, 1923). The technique of preparing soil dilution was same for all the microorganisms, except the dilution strength varied accordingly. Petridishes were incubated at 27 ± 2°C .The colony forming units (c.f.u.) of with the varying dilution strength of total fungal population(10^{-3}), total bacterial population(10^{-6}), actinomycetes(10^{-5}), *Trichoderma* spp. (10^{-3}) and *Pseudomonas fluorescens* (10^{-6}) were recorded,respectively.

The population count of fungi were estimated by using peptone dextrose rose bengal agar medium (Martin, 1950), bacteria by using nutrient agar medium , actinomycetes by using starch ammonium agar medium (Kuznetsov and Arjunarao, 1972), *Trichoderma* spp, by selective media(Elad and Chet,1983) and *Pseudomonas fluorescens* by specific King's B medium (King *et al.,* 1954).

RESULTS AND DISCUSSIONS

The effects of soil solarization on microbial populations present in solarized and non-solarized plots integrated with organic amendments and bioagents have been studied. We have considered only microbiological changes at two stages i.e. just after solarization and after 30 days of solarization in rhizosphere and non-rhizosphere soil. The population count of total fungi, bacteria, actinomycetes, *Trichoderma* spp. and *Pseudomonas fluorescens* were estimated by the soil dilution method.

The results of the total estimated population (c.f.u. × 10^{-3}/g soil) of fungi are recorded in Table 1 and Fig. 1a and 1b. It is evident from the results that while in non-solarized treatment the populations ranged from 31 to 56 × 10^{-3}/g soil, the counts in solarized treatments were 13 to 27 × 10^{-3}/g soil. As against the population of 13 to 27 × 10^{-3}/g soil immediately after solarization changed to 22.33 to 39.40 × 10^{-3}/g soil in rhizosphere soil and 31 to 47.33 × 10^{-3}/g soil in non-rhizosphere soil.

Bacteria constitute the maximum population among the soil microflora. Solarization for 8 weeks reduced their counts (c.f.u.) by almost 50 per cent (Table 2). In non-solarized plots the number recorded ranged from 70 to 99.56 × 10^{-6}/g soil. Contrary to it under solarized condition, the numbers ranged only from 40 to 61.23 × 10^{-6}/g soil. The populations in rhizosphere soil is 54.00 to 66.26×10^{-6}/g soil and in non-rhizosphere soils ranged from 61.66 to 87.33 ×10^{-6}/g soil.

After the population dynamics of fungi and bacteria, actinomycetes, occupy soil's ecological niche as a component of soil microbiota and food chain. In this context, the effects of solarization with or without integration with organic amendments and bioagents were studied. The results so obtained are given in Tables 3.

The results clearly revealed that solarization for 8 weeks reduced the counts (c.f.u.) of actinomycetes significantly over the counts, recorded in non-solarized soil. The counts (c.f.u.) in solarized conditions ranged from 3.66 to 8.33 × 10^{-5}/g soil. Contrary to it, under non-solarized conditions, the counts ranged from 6.83 to 13.33 × 10^{-5}/g soil when the populations were estimated after 30th day in non-rhizosphere soil, the

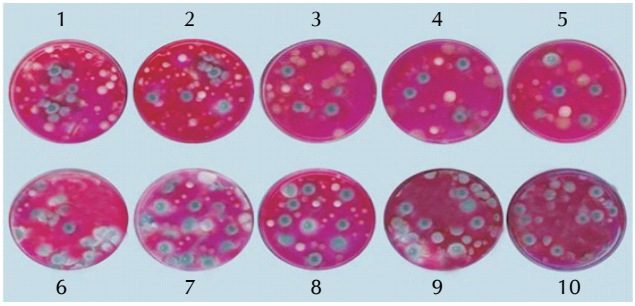
Table 1: Effect of soil solarization integrated with organic amendments and bioagents on population dynamics (cfu × 10^{-3}) of total fungi

S. No.	Treatment	Rate of application per plot	Average* number of colonies × 10^{-3} Just after soil solarization (8 weeks)	30 days after soil solarization in rhizosphere soil	30 days after soil solarization in non-rhizosphere soil
1.	Solarized soil	-	19.33	22.33	31.00
2.	Solarized soil + FYM	FYM-2 kg	21.66	34.00	39.00
3.	Solarized soil + PM	PM-2 kg	18.66	38.00	45.00
4.	Solarized soil + *T. harzianum*	*T. harzianum*-6 gm	13.00	31.00	36.00
5.	Solarized soil + *P. fluorescens*	*P. fluorescens*-6 gm	27.00	39.40	47.33
6.	Non-solarized soil + FYM	FYM-2 kg	39.00	55.23	64.66
7.	Non-solarized soil + PM	PM-2 kg	56.00	68.14	77.66
8.	Non-solarized soil + *T. harzianum*	*T. harzianum*-6 gm	51.66	64.00	73.56
9.	Non-solarized soil + *P. fluorescens*	*P. fluorescens*-6 gm	35.00	50.00	61.00
10.	Non-solarized soil (check)	-	31.00	49.00	58.56
	SEm ±		2.97	3.25	4.41
	CD at 5%		8.76	9.59	13.01
	CV (%)		16.40	12.11	17.41

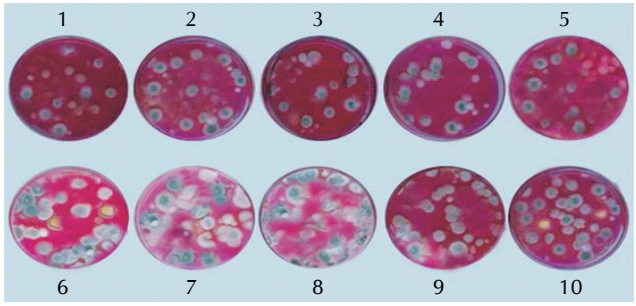
* Mean of 3 replications, Organic Amendment - FYM = Farm yard manure, P.M. = Poultry manure, Biocontrol Agent - *T.harzianum* - *Trichoderma harzianum* *P.fluoresecens* - *Pseudomonas fluorescens*

population recovered, upto to some extent and was almost similar to what was recorded after solarization. The effect of organic amendments and bioagents too was only marginal. In rhizosphere soils of the crop raised, the population was slightly higher as compared to counts in non-rhizosphere soils.

The fungal bioagent *Trichoderma* spp. are basically soil saprophytes and constitute a sizeable population of fungal flora. The results so obtained are given in Tables 4 and Fig. 2a and 2b. In non-solarized plots the counts (c.f.u.) of this antagonist ranged from 24.44 to 41.44 $\times$ 10^{-3}/g soil. Contrary to it, solarization reduced population very significantly as the counts recorded ranged only 15.44 to 28.22 $\times$ 10^{-3}/g soil. However, the population at 30th day after raising a nursery crop recovered fully, rather increased also. Against the counts of 15.44 to 28.22 $\times$ 10^{-3}/g soil, the counts at 30th day ranged 29 to 39.33 $\times$ 10^{-3}/g soil in non-rhizosphere soil and 24.33 to 31.66 $\times 10^{-3}$/g soil in rhizosphere soil. After 30th day, in treatments involving no solarization, the population of *Trichoderma* spp. was significantly higher as compared to treatments involving solarization. It is because of the fact that initial population of this antagonist already existed in soil. Integration of organic amendments and bioagents, possibly increased non-rhizosphere competence of the antagonist as the population in integrated plots were invariably higher.

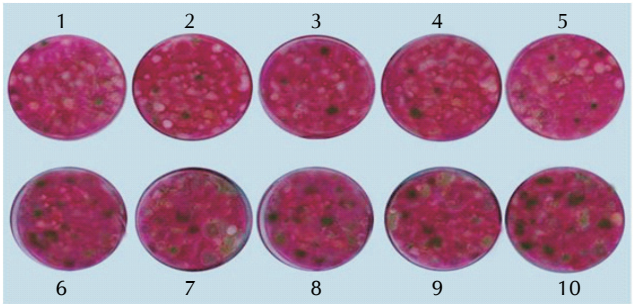


(a) Just after soil solarization

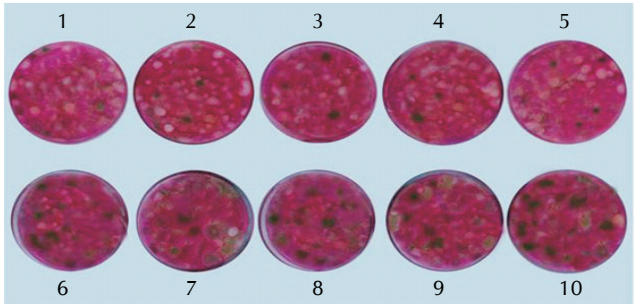


(b) 30 days after soil solarization in non-rhizosphere soil

Figure 1: Effect of soil solarization with organic ammendments and bioagents on population dynamics (cfu $\times$ 10^{-3}) of total number of fungi in different treatments (1-10)



(a) Just after soil solarization



(b) 30 days after soil solarization in non-rhizosphere soil

Figure 2: Effect of soil solarization integrated with organic ammendments and bioagents on population dynamics (cfu $\times$ 10^{-3}) of total number of *Trichoderma* spp. in different treatments (1-10)

Table 2: Effect of soil solarization integrated with organic amendments and bioagents on population dynamics (cfu $\times$ 10^{-6}) of total number of bacteria

S. No.	Treatment	Rate of application per plot	Average* number of colonies $\times$ 10^{-6} Just after soil solarization (8 weeks)	30 days after soil solarization in rhizosphere soil	30 days after soil solarization in non-rhizosphere soil
1.	Solarized soil	-	40.00	54.00	61.66
2.	Solarized soil + FYM	FYM-2 kg	55.00	63.00	87.33
3.	Solarized soil + PM	PM-2 kg	61.23	66.26	72.23
4.	Solarized soil + *T. harzianum*	*T. harzianum*-6 gm	55.33	66.00	84.44
5.	Solarized soil + *P. fluorescens*	*P. fluorescens*-6 gm	45.00	59.44	74.96
6.	Non-solarized soil + FYM	FYM-2 kg	78.66	91.46	111.45
7.	Non-solarized soil + PM	PM-2 kg	89.00	99.46	114.50
8.	Non-solarized soil + *T. harzianum*	*T. harzianum*-6 gm	99.56	121.56	140.23
9.	Non-solarized soil + *P. fluorescens*	*P. fluorescens*-6 gm	70.00	80.00	90.44
10.	Non-solarized soil (check)	-	77.00	81.00	88.00
	SEm $\pm$		11.52	6.78	9.38
	CD at 5%		34.00	20.02	27.67
	CV (%)		23.76	24.75	14.55

* Mean of 3 replications, Organic Amendment - FYM = Farm yard manure, P.M. = Poultry manure, Biocontrol Agent - *T.harzianum* - *Trichoderma harzianum* *P.fluoresecens* - *Pseudomonas fluorescens*

Table 3: Effect of soil solarization integrated with organic amendments and bioagents on population dynamics (cfu × 10^{-5}) of total number of actinomycetes

S. No.	Treatment	Rate of application per plot	Average* number of colonies × 10^{-6} Just after soil solarization (8 weeks)	30 days after soil solarization in rhizosphere soil	30 days after soil solarization in non-rhizosphere soil
1.	Solarized soil	-	7.33	8.33	7.90
2.	Solarized soil + FYM	FYM-2 kg	8.33	9.33	9.00
3.	Solarized soil + PM	PM-2 kg	3.66	6.00	5.21
4.	Solarized soil + *T. harzianum*	*T. harzianum*-6 gm	4.33	9.33	9.21
5.	Solarized soil + *P. fluorescens*	*P. fluorescens*-6 gm	6.33	8.31	16.90
6.	Non-solarized soil + FYM	FYM-2 kg	11.66	13.89	12.20
7.	Non-solarized soil + PM	PM-2 kg	9.00	12.23	11.23
8.	Non-solarized soil +	*T. harzianum*	*T. harzianum*-6 gm	6.83	9.66 7.46
9.	Non-solarized soil + *P. fluorescens*	*P. fluorescens*-6 gm	10.33	14.32	12.33
10.	Non-solarized soil (check)	-	13.33	14.33	14.00
	SEm ±		1.61	2.11	1.84
	CD at 5%		4.76	6.24	5.45
	CV (%)		34.98	2.11	32.14

* Mean of 3 replications, Organic Amendment - FYM = Farm yard manure, P.M. = Poultry manure, Biocontrol Agent - *T.harzianum* - *Trichoderma harzianum* *P.fluoresecens* - *Pseudomonas fluorescens*

Table 4: Effect of soil solarization integrated with organic amendments and bioagents on population dynamics (cfu × 10^{-3}) of total number of *Trichoderma* spp.

S. No.	Treatment	Rate of application per plot	Average* number of colonies × 10^{-6} Just after soil solarization (8 weeks)	30 days after soil solarization in rhizosphere soil	30 days after soil solarization in non-rhizosphere soil
1.	Solarized soil	-	19.44	24.33	29.66
2.	Solarized soil + FYM	FYM-2 kg	27.00	29.33	39.33
3.	Solarized soil + PM	PM-2 kg	28.22	31.66	36.33
4.	Solarized soil + *T. harzianum*	*T. harzianum*-6 gm	15.44	25.66	29.00
5.	Solarized soil + *P. fluorescens*	*P. fluorescens*-6 gm	16.60	26.66	33.00
6.	Non-solarized soil + FYM	FYM-2 kg	41.44	49.33	58.00
7.	Non-solarized soil + PM	PM-2 kg	32.22	39.00	52.00
8.	Non-solarized soil + *T. harzianum*	*T. harzianum*-6 gm	31.56	37.33	51.00
9.	Non-solarized soil + *P. fluorescens*	*P. fluorescens*-6 gm	24.44	30.33	46.11
10.	Non-solarized soil (check)	-	28.56	32.33	48.56
	SEm ±		4.85	2.85	2.62
	CD at 5%		14.33	8.41	7.73
	CV (%)		27.12	16.74	15.56

* Mean of 3 replications, Organic Amendment - FYM = Farm yard manure, P.M. = Poultry manure, Biocontrol Agent - *T.harzianum* - *Trichoderma harzianum* *P.fluoresecens* - *Pseudomonas fluorescens*

Table 5: Effect of soil solarization integrated with organic amendments and bioagents on population dynamics (cfu × 10^{-6}) of total number of *Pseudomonas fluorescens*

S. No.	Treatment	Rate of application per plot	Average* number of colonies × 10^{-6} Just after soil solarization (8 weeks)	30 days after soil solarization in rhizosphere soil	30 days after soil solarization in non-rhizosphere soil
1.	Solarized soil	-	66.33	57.33	57.00
2.	Solarized soil + FYM	FYM-2 kg	60.00	56.83	56.21
3.	Solarized soil + PM	PM-2 kg	68.00	65.00	64.23
4.	Solarized soil + *T. harzianum*	*T. harzianum*-6 gm	61.00	51.00	50.99
5.	Solarized soil + *P. fluorescens*	*P. fluorescens*-6 gm	52.00	49.23	48.88
6.	Non-solarized soil + FYM	FYM-2 kg	31.66	31.33	31.00
7.	Non-solarized soil + PM	PM-2 kg	40.33	37.00	36.66
8.	Non-solarized soil + *T. harzianum*	*T. harzianum*-6 gm	37.00	36.21	35.66
9.	Non-solarized soil + *P. fluorescens*	*P. fluorescens*-6 gm	36.21	35.21	34.21
10.	Non-solarized soil (check)	-	35.00	34.21	33.21
	SEm ±		8.60	7.28	6.31
	CD at 5%		25.39	21.48	18.63
	CV (%)		30.65	28.11	21.74

* Mean of 3 replications, Organic Amendment - FYM = Farm yard manure, P.M. = Poultry manure, Biocontrol Agent - *T.harzianum* - *Trichoderma harzianum* *P.fluoresecens* - *Pseudomonas fluorescens*

The results clearly establish the fact that the count of microbial populations (of fungi, bacteria, actinomycetes and *Trichoderma* spp.) not only fully recovered but rather increased also in rhizosphere and non-rhizosphere soils, estimated after 30 days of solarization, at the time of normal cultivation of nursery crop. The population count of microbial populations in treatments with non-solarization also increased when compared to population recorded immediately after solarization. The population counts in solarization was invariably lower as compared to non-solarization treatments.

In the non-rhizosphere soil, the population count was increased. The increase was more pronounced in non-solarization than in solarization.

It is obviously due to thermal inactivation of these eliminated microflora as they could not tolerate the impact of increased temperature.

Similar observations have been recorded by several workers (Katan, 1981; Milevoj, 1989; Chaube and Singh ,1991; Elena *et al.,* 1997; Muhammad *et al.,* 1999; Wadi, 1999; Triki *et al.,* 2001; Khalaif, 2003; Shukla and Dwivedi, 2011; Gamliel and Katan, 2012; Sharma and Razdan, 2011; Anthony, 2013). Soil-solarization resulted in a significant reduction in the population of fungi, bacteria and actinomycetes in soil (Joshi *et al.,* 2009; Patel *et al*., 2009).

Apart form above, among the bacterial populations, the population of plant growth promoting rhizobacteria pseudomonas has invariably been recorded to be about 40 per cent of the total bacteria existing in soil.

The results of the effects of soil-solarization on counts of *Pseudomonas fluorescens* are given in Tables 5. It is evident from the data that solarization of soil for 8 weeks, did not reduce the counts of the group of bacteria rather, solarization increased the population in solarized soil. The average counts ranged from 31.66 to 40.33 $\times$ 10^{-6}/g in non-solarized soil contrary to it, under solarized conditions the counts ranged from 52 to 68 $\times$ 10^{-6}/g soil. Thus, there was significant increase in population. Population estimated after 30 days i.e. after raising a crop did not show any appreciable change from the counts recorded after solarization in the rhizosphere soil as well the counts were almost similar to what was recorded in non-rhizosphere soil. It is clear that the populations that survived the impact of solarization multiplied in due course of time to occupy the ecological niches that had fallen vacant. Such recoveries are made by the *Pseudomonas fluorescens* which are thermo-tolerant and thermo-sensitive. Such observation have been made by other workers (Gamliel and Katan, 1992; Gamliel and Stapleton, 1993a and b; Sastry and Chattopadhyay, 1999; Kumar *et al.,* 2001; Stevens *et al.,* 2003; Jayaraj *et al.,* 2007 and Bonanomi *et al*., 2008).

Soil incorporation of organic amendments and specifically poultry manure and FYM, significantly augmented the rhizosphere population of the marked *Pseudomonas fluorescens* strain (Jayaraj *et al.,* 2007). Rhizosphere population of introduced biocontrol agents gradually increased *Pseudomonas fluorescens* strain in solarized soils when compared to unsolarized control (Jayaraj and Radhakrishnan, 2008) Organic amendment significantly enhanced the organic matter content, the hydrolysis of fluorescein diacetate and the Pseudomonas population (Bonanomi *et al*., 2008).

Hence regarding the microbiological changes of naturally existing populations of fungi, bacteria, actinomycetes, *Trichoderma* spp. and *Pseudomonas fluorescens* were affected by solarization carried out for 8 weeks. The populations of all the microbiota except *P. fluorescens* decreased significantly. However, after 30 days i.e. after raising a nursery crop, the estimated population showed significant recovery. The population of *P. fluorescens* changed only marginally.

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