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Supriya Gupta
Department of Plant Pathology, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

RP Singh
Department of Plant Pathology, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Pankaj Rautela
Department of Plant Pathology, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Correspondence
Supriya Gupta
Department of Plant Pathology, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Effect of soil solarisation on survival of sclerotia and viability of antagonists under protected and natural cultivation

Supriya Gupta, RP Singh and Pankaj Rautela

Abstract
Soil borne pathogens persist in soil and cause damage to variety of crops. None of the disease control technique available presently can bring the level of soil sanitation below critical threshold to reduce seed and seedling disease. Solarization appears to be of major use in protected cultivation, as once a disease has introduced in protected structures it is very difficult to manage it. The study conducted under different polyhouse conditions for 45 days revealed that the survival of the *Sclerotinia sclerotiorum* decreases with increase in depth. Minimum survival of 20 per cent was found in controlled condition. There was a significant reduction in the viability of *Trichoderma harzianum* and *Pseudomonas fluorescence* at 10cm depth in controlled, naturally ventilated and outside conditions as compared to non solarized soils. The maximum reduction was found in outside solarized soil followed by naturally ventilated and minimum reduction was found in controlled polyhouse conditions at 10 cm depth. While there was no significant change found in the viability of both at 20 cm depth in controlled polyhouse.

Keywords: soil solarisation, natural cultivation, *Trichoderma harzianum*, *Pseudomonas fluorescence*

1. Introduction
Plants are the source of food for multitudes of macro and micro organisms in ecosystem. Diseases constitutes a major constraint in increasing the production, productivity and availability of food products. Soil borne pathogens constitute the first bio hostile element of the soil ecosystem that plants encounter during emergence and also at various stages of growth and development. In nurseries the crop suffers more due to several seed-seedling diseases, presumably caused by soil inhabitants and/or invaders (Esfahani, 1991) [6].

The control of such soil borne diseases have never been satisfactory, none of the disease control techniques available presently which can bring the level of soil sanitation below critical threshold to reduce seed and seedling disease (Chaube and Singh, 1991) [2]. The recommended routine sanitation approaches that include crop rotation and soil disinfections with fumigants and other pesticides have become questionable and therefore restricted because of there incompatibility with the concept of sustainable agriculture. This necessitates the search for an alternative soil disinfection methodology. An innovative approach is soil Solarization (Chet, 1987) [3].

It gives beneficial effects on environmental sustainability as solarization process leaves no toxic residues in the environment. Soil solarization determines a temperature increase of 2 to 15˚C above the temperature of untreated soil. This effect is successful especially to control those plant pathogens and pests that are heat sensible and unable to survive at temperatures above 37–40 °C. Furthermore, soil pasteurization by solar heating gives positive effects against a broad number of seed weeds affecting crops cultivation and also results in an increased growth response (IGR) of plants (Katan,1981) [16]. Solarization targets only mesophyllic organisms, which include most plant pathogens and pests without destroying beneficial fungi and plant growth promoting bacteria (Stapleton and DeVay, 1982, 1984; Gamliel and Katan, 1991) [21, 20, 9].

Although soil solarization is effective in controlling pathogens situated in the upper soil layers, its efficacy declines with soil depth. Incorporation of additional suppressive factors is often necessary for improving the efficacy of solarization. Combining biological control with solarization could ideally benefit the proliferation and colonization of biocontrol agents and thereby induce soil suppressiveness to plant pathogens.

Polyhouse farming is an alternative technique in agriculture gaining foothold in India. It is becoming increasingly popular both in temperate and tropical region. It reduces dependency on rainfall and makes the optimum use of land and water resources.

It enables cultivation of off-season crops regular, thus fetching the farmer a higher price (Singh and Malhotra, 2012) [19]. Protected structures on one hand provide ambient growing conditions to the plant, on the other hand this condition is congenial to the plant pathogens also. Though protected farming has advantage that pathogen do not enter easily from outside but once a pathogen has introduced, it is very difficult to manage. Control of particularly soil borne plant pathogen under polyhouse is a challenge, as traditional practices like crop rotation, fallow, mixed cropping etc. usually cannot be applied. Solarization appears to be of major use in greenhouse culture. The ability of greenhouse operators to close up greenhouses during the hot summer months allows higher solarization temperatures than achievable in treatment of open fields. As with use in greenhouses, these are ideal niches for solarization, since individual areas to be treated are small, soil temperature can be greatly increased, the cost of application is low, the value of the plants produced is high, and the production of disease free planting stock is critical for producing healthy crops (Stapleton, 1997) [22]. Lot of work have been done on soil solarization in open field condition but under protected cultivation detailed scientific information is lacking in india, though it is being practiced by most of the growers.

Material and methods

In present study field investigations were carried out at Precision Farming Development Centre (PFDC) and laboratory experiments in the Department of Plant Pathology of G.B. Pant University of Agriculture and Technology, Pantnagar.

Solarization Technique

Solarization was done in open field condition as well as inside the polyhouses. Two different types of polyhouse structures i.e. controlled and naturally ventilated were used. The length and width of polyhouse was 30 feet x 12 feet respectively having side height was 8 feet and the central height of the polyhouse was 11 feet. The cladding material was of 150 GSM, UV stabilised and cross laminated polyfilm. Naturally ventilated polyhouse was made up of bamboo structure. Controlled polyhouse was made up of GI structure and mechanically ventilated to mantain desired temperature and moisture conditions.

Before mulching the soil with polyethylene sheet, pre-irrigation was done to ensure enough moisture during the period of solarization. After that it was covered with 25 µm thick transparent polyethylene sheet to maintain air tight condition. After covering the plots with polyethylene sheets, the edges of polyethylene sheet were sealed in furrows and burried in the soil. Firm polyethylene mulch was insured to prevent any leakage of heated air, gases, moisture etc., as are essential for successful solarization. Care was also taken to avoid damage to polyethylene sheets. The damage of any kind was repaired immediately. Solarization was done for 43 days between 11 June to 23 July, 2011.

Soil Sampling

Soil samples were taken from each conditions before and after solarization for physio-chemical analysis. Before analysis, the samples were sieved to remove plant debris, thereafter it was air dried for 18-25 hrs. For microbial population studies, samples were analysed the same day, but for physio-chemical properties it was stored in plastic bags at low temperature and subsequently analysed in the Department of Soil Science.

Effect of Soil solarization on Viability of Sclerotia

For studying the effect of soil solarization on viability of soil borne pathogens, two sclerotia forming pathogens namely *Sclerotium rolfsii* and *Sclerotinia sclerotiorum* were choosen. Sclerotia of *Sclerotinia sclerotiorum* was collected from heavily infected Capsicum plants grown under polyhouse. Sclerotia of *Sclerotium rolfsii* was produced under laboratory conditions on petriplates containing PDA.

Ten Sclerotia of *Sclerotium rolfsii* and five sclerotia of *Sclerotinia sclerotiorum* was packed in nylon bags and placed at a depth of 10 and 20 cm with the help of plastic rope in soil during the period of solarisation. Three replication of each were maintained. After solarization, bags were taken out, Sclerotia were surface sterilized with sodium hypochlorite (1%) for thirty seconds to one min followed by two to three washing with sterilized distilled water and then placed on sterilized blotter paper to remove excess moisture. Sterilized sclerotia were kept aseptically on Petri plates preceeded with potato dextrose agar (PDA). The inoculated Petriplates were incubated at 23±1 °C in BOD incubator to test their viability.

Effect of Soil Solarization on Antagonists

To see the effect of soil solarization on beneficial soil microorganisms fungal antagonist – *Trichoderma harzianum* and bacterial antagonist- *Pseudomonas fluorescen*s were choosen under the present study, Commercial formulations of bioagents developed by the Department of Plant Pathology, G.B. Pant university of agriculture and technology, Pantnagar namely PBAT-1(*Trichoderma harzianum*) and PBAP-2(*Pseudomonas fluorescen*s) were taken in the present study. The original *cfu* of both PBAT-1 and PBAT-2 were 2×10^8. One Hundred gram of freshly prepared talk based formulations were packed in cloth bag followed by nylon bags and incorporated in soil at depths of 10 and 20 cm with the help of plastic rope. Three replications of each bioagents were maintained during the period of solarisation. The packets were taken out after solarization and then cleaned and air dried and their viability were checked by counting cfu on selective media.

Populations of bioagents *Trichoderma harzianum*, dilution plate method using selective media described by Elad and Chet (1983) [4] was used. Population of *Pseudomonas fluorescens* was determined by employing dilution plate method using King's B medium (King *et al.,* 1954) [17]. A dilution of 1: 10^5 and 1: 10^6 was prepared from 1 g air dried Trichoderma formulation (PBAT-1) and 1 g *Pseudomonas fluorescens* formulation PBAT-2 respectively in sterilized tap water. One ml of sample was transferred to sterilized petriplates with the help of sterilized pipette and 20 ml of the sterilized but cooled medium was poured over it. The plates were rotated gently to distribute and suspend sample uniformly in the medium. The plates were incubated at 27± 2°C for three days before reading the observations. Number of colonies were counted with the help of Queback dark field colony counter.

Result and Dissscussion

Effect of Solarization on Survival of Sclerotia

a. *Sclerotinia sclerotiorum*

Assesment of viability just after solarization revealed that solar heating reduced the propagules of *Sclerotinia sclerotiorum* significantly in controlled polyhouse condition when compared to non solarized soil at different depths. The survival of the pathogen decreases with increase in depth. In

outside non solarized, outside solarized and naturally ventilated polyhouse survival was found to be 80 per cent while in controlled condition only 20 per cent propagules survived (Table-1). At 10 and 20 cm depth there was no recovery of pathogen in controlled, naturally ventilated and outside solarized soils whereas in non solarized soils 80 per cent and 60 per cent survival of pathogen was found at depth of 10 and 20 cm respectively. There was a reduction of 60 per cent propagules in controlled, semi controlled, outside solarized soils as compared to non solarized. Similar results were found by Ferraz *et al.* (2003) [7] have reported that Solarization killed sclerotia of *Sclerotinia sclerotiorum* at 5cm,10cm and 30cm depths.

b. Sclerotium rolfsii

Changes in the population of another versatile fungal pathogen, *S. rolfsii* due to prolonged solar heating is given in the Table 1. The data reveals that there was a significant decrease in the population of *S. rolfsii* at 10 and 20 cm depth in controlled polyhouse conditions, semi controlled, outside solarized and non solarized conditions. The survival of pathogen was found maximum at surface. Thirty per cent survival of propagules were found in non solarized condition followed by 20 per cent in outside solarized, 16.66 per cent in controlled and 10 per cent in naturally ventilated condition. There was no survival found at 10 and 20 cm in naturally ventilated and 10 cm in controlled conditions whereas survival of 6.66 per cent and 3.33 per cent at 10cm depth and 26.66, 6.60 at 20 cm depth at non solarized and outside solarized conditions respectively. After artificial inoculation of soil with sclerotia of *S. rolfsii* at different depths following solarization, 85-100 per cent decreased viability of sclerotia have been reported by many workers under open field conditions. (Elad *et al.*, 1980 [5]; Grinstein *et al.*, 1979 [10]; Horowitz, 1980 [12]; Jacobsohn, 1980 [13]; Stevens *et al.*, 2003 [23]).

Effect of Soil Solarization on Population of Antagonists

One of the mechanism advocated to be operative in the management of soil borne plant diseases is development of antagonistic association which regulates the activities of concerned pathogen. In soil solarization as well, these mechanisms have been implicated to be operational. It was in this context, that the population of two well known antagonists *Trichoderma harzianum* and *Pseudomonas fluorescens* were studied and results are described in the following sub sections.

Table 1: Effect of soil solarization on survival of *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* at different depths

Treatment	Survival of *Sclerotinia sclerotiorum* (%)			Survival of *Sclerotium rolfsii* (%)		
	Surface	10 cm	20 cm	Surface	10 cm	20 cm
Controlled Polyhouse Solarised	20	00	00	16.66	0.00	3.33
Naturally ventilated polyhouse solarized	80	00	00	10	0.00	0.00
Outside solarised	80	00	00	20	3.33	6.66
Outside non solarized	80	80	60	30	6.66	26.66
CD (P- 0.05) Depth	7.69			4.21		
Treatment	8.88			4.86		
Interaction	15.38			8.42		

a. ***Trichoderma harzianum***

Among the fungal species known for antagonists potential, *Trichoderma spp.* has been a subject of indepth studies in recent years. Effect of solarization on viability of antagonists is naturally a matter of intrest. Population of *Trichoderma harzianum*, assessed on selective media are presented in Table 2. It is evident from the table there was a significant reduction in the viability of *Trichoderma harzianum* at different depth in controlled, naturally ventilated and outside solarised soil was observed as compared to non solarized soils. The *cfu* ml^{-1} of antagonist at dilution of 10^5 was 9.83, 1.50, 4.50, 8.66 in controlled, semicontrolled, outside solarized and non solarized at 10 cm depth whereas higher *cfu* of 11.83, 3.66 was found in controlled and naturally ventilated solarized soil at 20 cm depth. Population of *T. harzianum* reduced at 10cm and 20cm depth. The decline in the population of *Trichoderma* in soil following solarization are contradictory to the reports of Horiuchi (1984) [11], Katan (1987 [14], 1983 [15]) Rubin and Benjamin (1984) [18], Stapleton and Devay (1984) [20] which indicate that constant soil moisture provided by plastic sheet provide better conditions for activity of natural antagonists in soil. The results of present investigation are in accordance with the findings of Esfahani, 1991[6] which indicates that *Trichoderma* do not tolerate heat generated by polyethylene mulching.

b. ***Pseudomonas fluorescence***

Results of study of soil solarization on viability of *Pseudomonas fluorescence* in solarized soil is given in the table 2. The results clearly reveled that there is a significant difference in population of *Pseudomonas fluorescence* at depth of 10 and 20 cm. The maximum reduction was found in outside solarized soil followed by naturally ventilated and minimum reduction was found in controlled polyhouse conditions at 10 cm depth. At 20 cm depth there was no significant difference in *cfu* ml^{-1} at dilution of 10^{-7} of *P. fluorescence* in all treatments. The results were in confirmation with the work done by Bueno *et al.* (2003) [1] which revealed that soil solarization reduced the community of *Pseudomonas fluorescence* to undetectable levels. The results contradict the findings of Gamliel and Katan (2005) [8] who observed that population densities of fluorescent pseudomonads were increased up to 130-fold in the rhizosphere of plants in solarized soils, although these bacteria are heat sensitive.

Table 2: Effect of soil solarization on viability of Trichoderma harzianum and Pseudomonas fluorescence at different depth

Treatment	Trichoderma harzianum (10^5)		Pseudomonas fluorescence (10^7)	
	10 cm	20 cm	10 cm	20 cm
Controlled polyhouse solarized	9.83	11.83	183.33	25.33
Naturally ventilated polyhouse Solarised	1.50	3.66	28.16	51.66
Outside solarised	4.50	3.33	17.88	38.00
Outside non solarized	8.66	6.66	201.83	35.83
CD (P- 0.05) Depth	I.33		17.76	
Treatments	1.89		25.11	
Interaction	2.67		35.52	

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