Ann. For. Res. 53(2): 117-126, 2010

ANNALS OF FOREST RESEARCH
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Solarization of nursery soil induces production of fruit bodies of mushrooms and enhances growth of tropical forest tree seedlings

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Verma R. K., Thakur A. K., Turkane D., Rajput P. S. 2010. Solarization of nursery soil induces production of fruit bodies of mushrooms and enhances growth of tropical forest tree seedlings. Ann. For. Res. 53(2): 117-126, 2010.

Abstract. The aim of this work was to find out the effect of soil solarization on microbial population and its effect on growth of two species of tropical forest trees. For this purpose, solar heating of nursery seedbeds (1 x 5m) was done during April-May 2009 for one month, by application of a thin clear sheet of polyethylene. The top soil (5 inches) consists of a mix of loam soil, sand and farm yard manure in 2:1:0.5 ratios (v/v). Temperature variations were recorded daily for a period of one month, at 2 depths, (5 cm and 10 cm). Maximum differences in temperature between solar treatment and control was recorded as high as 12.1° C at 5 cm and 9.1° C at 10 cm depth. After one month, population of *Aspergillus, Fusarium, Penicillium, Rhizopus* and nematodes were completely eliminated from upper 5 cm depth, although population of AM fungi, bacteria and *Trichoderma* were reduced, but not completely eliminated. Seedlings of *Gmelina arborea* Roxb. and *Tectona grandis* Linn.f. were raised through seeds on treated and control beds. After three months, the production of fruit bodies of mushrooms, namely *Amanita populiphila* Tullos & E. Moses*, Lepiota longicauda* Henn. and *Scleroderma* sp. were observed. It was noticed that these mushrooms only appeared on treated soil with white mycelial growth in rhizosphere under fruit bodies. *Lepiota longicauda* produced the maximum number of fruit bodies on teak seedbeds followed by *Scleroderma* sp. on *G. arborea* seedbeds. Due to solar heating there was 23.9% increase in plant height and 22.1% increase in collar diameter of *G. arborea* seedlings, where as 17.4% increase in plant height and 9.8% increase in collar diameter in case of *T. grandis,* as compared to control seedlings.
Keywords: *Gmelina arborea*, plant growth, soil disinfection, soil microbes, solar heating, *Tectona grandis*

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Manuscript received August 27, 2009; revised October 15, 2010; accepted October 19, 2010; online first December 30, 2010.

Introduction

During production of seedlings in nursery, soil-borne plant pathogens create serious problems to nursery growers, especially when the soil is sick. The soil pathogens can be removed by disinfecting the soil. There are three methods to disinfect sick soil: (i) "fumigation", (ii) "steaming" and (iii) "solarization". The first method requires hazardous chemical, like methyl bromide, while the second one requires lot of infra structure and cost. The third one is eco-friendly and cost low than the former methods. Solarization (also referred to as solar heating of the top soil) is a method of soil disinfestations, first described by J. Katan and coworkers (Katan et al. 1976) for controlling pathogens and weeds. It is achieved by covering (mulching or tarping) the soil with transparent clear polyethylene sheets (0.3-5 mil thick) to trap solar radiation to heat it. It is a hydrothermal process that can be used in moist soil covered with clear plastic tarp and exposed to direct sunlight in tropical and temperate regions. Solarization catches solar radiation and thereby heats the soil to raise temperature sufficiently to suppress or eliminate pests and pathogens. It can be effective against a broad spectrum of diseases, fungi, weeds, nematodes, insect pests and most soil borne bacteria (Katan 1981). Solarization also causes complex changes in the biological, physical and chemical properties of the soil, that improve plant development, growth quality and yield for up to several years (Freeman et al. 1990, De Vay et al. 1990, Stapleton & De Vay 1982). In areas with suitable climate, solarization with lethal or sub lethal fumigation or biological control is used to provide an effective substitute to chemical fumigation (Hartiz et al. 1993, Raj & Sharma 2008). It was used as a tool to control root pathogens along with management of ectomycorrhizal fungi for production of seedlings of forest trees (Annesi & Motta 2007, Perrin et al. 1998, Pinkerton et al. 2002, Salerno et al. 2000, Soulas et al. 1997); it was also reported to increase root nodulation, colonization by arbuscular mycorrhizal (AM) fungi and yield of cowpea (Nair et al. 1990). Solar heating affects a wide range of soil micro-organisms (Katan 1987), but very little is known about the effect of it on mycorrhizal fungi. A few reports exist of side effects of solarization on AM fungi. Contradictory results have been reported, but most suggest that heating the soil does not damage native AM fungi and can enhance mycorrhization and growth of plants in solarized soil (Afek et al. 1991).

Wilt and root rot diseases caused by *Fusarium* spp. attacked seedlings of *Gmelina arborea* Roxb. and *Tectona grandis* Linn. f. in seedbeds and hindered seedling production in nursery (Joshi & Jamaluddin 2007). Therefore, the present work has undertaken to study the effect of solarization of seedbeds on growth of seedlings of two important tropical tree species of central India.

Materials and methods

Design of experiment. The experiment was conducted in randomized complete block design (RCBD) with 4 blocks in each treatment.

Location of experiment. The experiment was conducted at research experimental area of Forest Pathology Division, Tropical Forest Research Institute, Jabalpur, Madhya Pradesh, India (Figure 1) which is located on N $23^0$06'074" E $79^0$59'386", elevation above sea level 415 m. The place experiences warm weather during April-June with average temperature ranges between 41° C-21° C during these months and 27° C-8° C during the winters (November-February). The annual rainfall is 1386 mm, and monsoon arrives at this place in the beginning of July and prolong up to September.

Laying of tarp. Forest nursery seedbeds 1 x 10 m were chosen for solarization. The beds were irrigated to the field capacity and half of each seedbed (1 x 5 m) was covered with clear

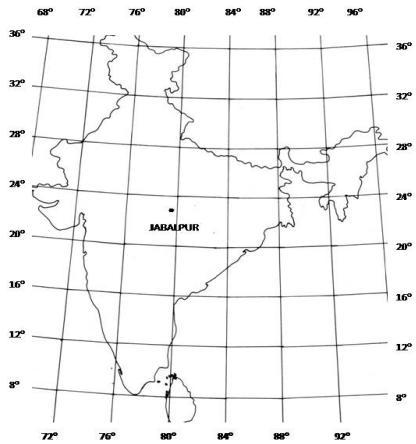


Figure 1 Map showing location of experiment

polyethylene sheet (commercial grade, 0.11 mm thick). Edges of sheets were buried in soil up to 5"-6", to make it air tight from all corners. Any puncture in the sheet due to rise in temperature or due to any injury was promptly closed with rigid tape, to check loss of heat, water vapor, CO_2 etc. The beds were watered as and when required to maintain sufficient moisture in the soil.

Measurement of soil temperature. Soil thermometers were buried at 5 cm and 10 cm depth in both solarized and non-solarized (control) seedbeds. The temperatures were recorded in afternoon, between 2-3 PM daily, for 30 days during May-June, 2009.

Determination of microbial population. Soil samples were collected from experimental seedbeds, just before laying of tarp and after 30 days of solarization; population of bacteria and fungi were determined by dilution plate method (Warcup 1950, 1955). Spores of AM fungi and the number of nematodes in solarized and control soil were counted under the stereo zoom microscope, after isolating them by wet sieving, sucrose centrifugation and floatation method as described by Sylvia (1994).

Raising of seedlings. Seedlings of *G. arborea* and *T. grandis* were raised from locally collected seeds on both, solarized and control seedbeds. Seeds of above mentioned tree species were sown on half portions (1 x 2.5 m) of each treated and control seedbed.

Determination of population of mushroom and recording of growth data of seedlings. Fruit bodies of mushrooms were observed and counted in both treated and control seedbeds of *Gmelina arborea* and *Tectona grandis*. Photos of mushrooms fruit bodies were taken with a digital camera (make Sony, 8.3 mega pixel) while photomicrographs were taken with the help of Leica digital camera attached to advanced research microscope model DMRBE. Plant heights and diameters at collar region were recorded after three months of seed sowing.

Statistical analysis. Mean and mode of temperature data were calculated. Data on microbial population was subjected to analysis of variance (ANOVA) and means were separated by using Duncans' Multiple Range Test when F values were found significant at $p = 0.05$.

Results

Soil temperature regime. Maximum temperature of solar heated soil at 5 cm depth has gone up to 61.2° C on June 6, 2009 only once during the entire period of experiment followed by 60.8° C one time, maximum frequency of temperature 56.2° C was 3. Maximum difference in soil temperature between treated and control soil was 12.1° C and this was obtained only once, followed by 11.8° C and 10.8° C while 10.3° C has the maximum frequency 6 during the entire period. The maximum frequency of difference in temperature was as high as 6 for 10.3° C, followed by 4 for 10.6° C and 3 for 10.4° C and 9.8° C. The mean difference in temperature during the study period was 10.3° C (Table 1).

At 10 cm depth the maximum temperature recorded during the study was 51.6° C. The maximum difference of temperature was 9.1°

C and the minimum was 4.0° C. The mean difference in temperature was 5.7° C (Table 1).

Microbial population. Soil bacteria had the maximum population in 5-10 cm soil depth, which sharply decreased by 4.13 times after solar treatment, while it was decreased by 4.67 times in 0-5 cm depth (Table 2).

The population of *Fusarium solani* (Mart.) Sacc., *F. oxysporum* Schltdl., *Penicillium* and *Rhizopus* spp. were totally eliminated after mulching at both depths. However, population of *Aspergillus* totally eliminated at 0-5 cm depth and reduced 53.2 times at 5-10 cm depth (Table 2).

Population of *Trichoderma* spp. was not eliminated totally after soil heating; however, it was reduced 1.3 times in soil collected from 0-5cm depth and 2.2 times in 5-10 cm depth (Table 2).

Population of AM fungi decreased 6 times but it was not totally eliminated at both depths (Table 3).

On the other hand population of soil nematodes totally eliminated at 0-5 cm depth and reduced by 24.7 times at 5-10 cm depth (Table 3).

Appearance of fruit bodies of mushroom. Fruit bodies of mushrooms started appearing after 45 days of seed sowing on solarized seedbeds only. They were observed

Table 1 Frequency (F) and variation in temperature in non-solarized (NS) and solarized (SS) beds (1 x 5 m) during May 17 to June 15, 2009

Frequency of soil temperature (°C) during solarization at two depths								Difference in soil temperature (°C) at two depths			
5 cm				10 cm				5 cm		10 cm	
NS	F	SS	F	NS	F	SS	F	Temp.	F	Temp.	F
44.6	1	54.8	1	38.4	1	44.3	1	9.3	1	4.0	1
44.7	1	55.0	1	39.0	1	44.5	2	9.6	1	4.2	1
45.2	1	55.1	1	39.2	1	45.0	1	9.7	1	4.3	1
45.3	2	55.4	2	39.8	1	45.1	1	9.8	3	4.6	2
45.4	2	55.6	2	40.0	1	45.5	1	10.0	1	4.9	1
45.5	1	55.7	2	40.1	1	45.7	1	10.1	2	5.1	1
45.6	1	55.8	2	40.2	3	46.0	2	10.2	3	5.2	4
45.7	1	56.2	3	40.3	1	46.2	2	10.3	6	5.3	1
45.8	1	56.3	1	40.6	1	46.3	1	10.4	3	5.4	1
46.2	2	56.4	1	40.8	2	46.4	1	10.5	1	5.5	2
46.3	1	56.8	2	41.1	1	46.8	1	10.6	4	5.6	1
46.4	2	57.2	1	41.3	1	47.0	1	10.7	1	5.7	2
46.5	1	58.0	1	41.4	3	47.2	2	10.8	2	5.8	2
46.6	1	58.1	1	42.0	1	47.4	1	11.8	1	5.9	1
46.8	1	58.2	1	42.2	1	47.6	2	12.1	1	6.0	2
47.4	2	59.0	1	42.3	1	49.0	2	-	-	6.3	1
47.5	1	59.1	1	42.5	1	49.4	1	-	-	6.5	1
48.6	2	59.2	2	43.3	1	49.6	2	-	-	6.6	1
48.7	1	60.1	1	44.0	1	49.8	1	-	-	7.2	1
48.8	1	60.4	1	44.3	1	50.0	1	-	-	7.3	2
49.4	1	60.8	1	44.6	1	50.2	1	-	-	9.1	1
49.6	1	61.2	1	45.0	2	51.2	1	-	-	-	-
49.8	1	-	-	45.4	1	51.6	1	-	-	-	-
50.2	1	-	-	45.6	1	-	-	-	-	-	-
Mean 46.8	-	57.2	-	41.7	-	47.4	-	10.3	-	5.7	-

for another one month and their populations were recorded from seedbeds of *Gmeline arborea* and *Tectona grandis*. Three species of mushrooms have been identified, viz. *Amanita populiphila* Tulloss & E. Moses (basidia 32-36 x 10.5-17.0 µm, spores hyaline, spherical to oval, smooth walled, 8.5-10.0 x 6-8 µm, Figures 2-7), *Lepiota longicauda* Henn. (pileus 6.5-10.5 cm diameter, stipe 5-10 cm long, basidiospores 7.5-8 x 4-5 µm, Figures 8-10) and *Scleroderma* sp. (basidia 8.5-19.0 x 5.5-7.0 µm, basidiospores 3.5-5.0 µm, Figures 11-13). Maximum population 8.25 per bed was recorded for *L. longicauda* in teak beds followed by 3.25 for *Scleroderma* sp. from seedbeds of *Gmelina arborea* (Table 4). *A. populiphila* and *Scleroderma* sp. are ectomycorrhizal mushrooms while *L. longicauda* is a saprophyte.

Recording growth data of seedlings. Data on height and diameter of seedlings at collar region was recorded after three months of seed sowing. Plant height was significantly higher in mulched seedbeds as compared to control. In the case of *Gmelima arborea,* seedlings grown on solarized seedbeds were 23.9% taller and 22.1% wider than seedlings of control seedbeds. In the case of *Tectona grandis* 17.4% increase in plant height and 9.8% increase in collar diameter were recorded (Table 5).

Discussion

Plant propagules, microorganisms, and micro fauna present in the soil start germination and

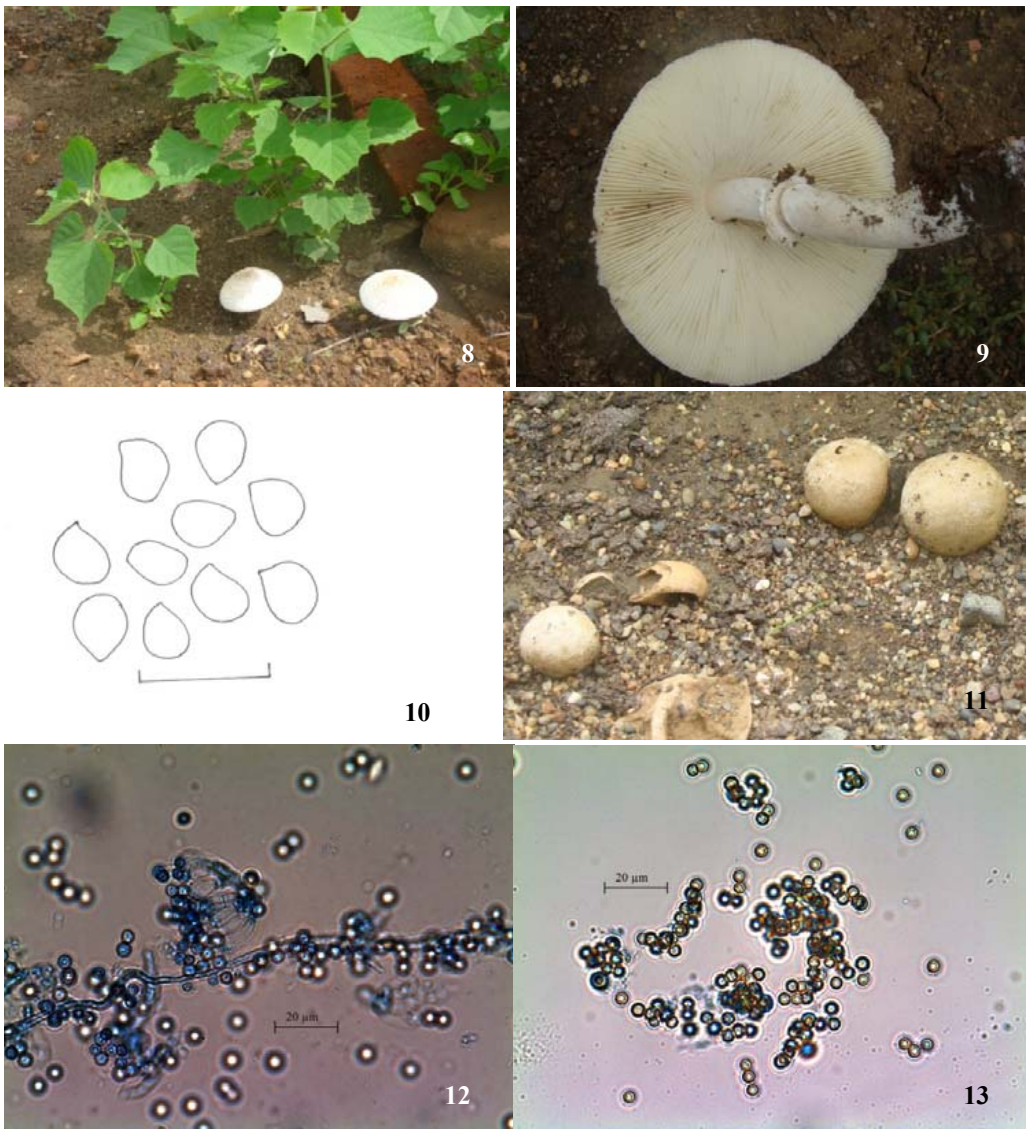
Table 2 Soil microbial population before and after solarization during May-June 2009 (means followed by the same letters for each organism are not statistically different at $p = 0.05$)

Name of microbe	Soil depth (cm)	Population (cfu g^{-1} x 10^3)	
		Before solarization	After solarization
Bacteria	0-5	71.00^{b}	15.20^{d}
	5-10	137.00^{a}	33.10^{c}
Aspergillus sp.	0-5	1.45^{b}	0.00^{c}
	5-10	2.13^{a}	0.04^{c}
Fusarium solani	0-5	0.15^{a}	0.00^{b}
	5-10	0.12^{a}	0.00^{b}
Fusarium oxysporum	0-5	0.24^{a}	0.00^{c}
	5-10	0.18^{b}	0.00^{c}
Penicillium sp.	0-5	0.39^{a}	0.00^{c}
	5-10	0.25^{b}	0.00^{c}
Rhizopus sp.	0-5	1.18^{b}	0.00^{c}
	5-10	4.12^{a}	0.00^{c}
Trichoderma sp.	0-5	0.16^{a}	0.12^{ab}
	5-10	0.11^{b}	0.05^{c}

Table 3 Population of AM fungi and nematodes in non-solarized and solarized soil (means followed by the same letters for each organism are not statistically different at $p = 0.05$)

Name of organism	Soil depth (cm)	Population ($50g^{-1}$soil)	
		Non-Solarized soil	Solarized soil
AM fungi	0-5	73^{a}	12^{c}
	5-10	43^{b}	07^{c}
Nematodes	0-5	81^{b}	0^{d}
	5-10	173^{a}	07^{c}

Figures 2-7 *Amanita populiphila*, 2-4 habit, 5-6 basidia with sterigmata attached with developing basid iospores, and 7 basidiospores



Figures 8-13 *Lepiota longicauda*. 8. Habit (emerging fruit bodies), 9. An uprooted fruit body showing stalk, vulva, annulus and gills. 10. Basidiospores, scale 20 µm. 11-13 *Scleroderma* sp. 11. Habit, 12. Mycelium, basidia, 13. Basidiospores.

multiplication due to moisture received before laying of the tarp and causes warming of a microclimate beneath the polyethylene sheet. During this process a lot of CO_2 is released due to respiration of germinating seeds, microorganisms, etc., which accumulated under the mulch. The humidity under the tarp increased due to evaporation of water. The CO_2 and water vapor create a green house effect under the mulch. The soil temperature increases due to these factors and goes up to lethal level (up to additional 12.1° C as compared to control in

Table 4 Appearance of fruit bodies of mushrooms in non-solarized and solarized seedbeds of *Gmelina arborea* and *Tectona grandis* after 3 months (means followed by the same letters are not statisti cally different at $p = 0.05$)

Name of Mushroom	*Gmelina arborea*		*Tectona grandis*	
	Non-solarized	Solarized	Non-solarized	Solarized
Amanita populiphila	0^{c}	0.5^{b}	0^{c}	1.00^{b}
Lepiota longicauda	0^{c}	2.50^{ab}	0^{c}	8.25^{a}
Scleroderma sp.	0^{c}	3.25^{a}	0^{c}	1.00^{b}

Table 5 Plant height and collar diameter of *Gmelina arborea* and *Tectona grandis* seedlings after three months grown on non-solarized and solarized soil mix (means followed by the same letters are not statistically different at $p = 0.05$)

Gmelina arborea		*Tectona grandis*	
Non-solarized	Solarized	Non-Solarized	Solarized
Height of seedlings (cm)			
29.15^{b}	36.12^{a}	16.70^{b}	19.61^{a}
Collar diameter of seedlings (cm)			
1.54^{b}	1.88^{a}	1.54^{b}	1.69^{a}

the present study), especially in the upper 5cm depth (Table 1) to kill the microorganisms.

In California, at 5 cm the temperature of tarped soil was recorded as high as 60° C (Katan 1981). In Florida at depth of 5, 15 and 25 cm, temperature of 49.5° C, 46.0° C and 41.5°C, respectively, was recorded in solarized soil (Chellemi et al. 1994). Pandey and Pandey (2004) reported 6-10°C increase in soil temperature at different moisture regimes. Studies conducted at Akola, India during May-June at 5 and 15 cm soil depths for 25 days, revealed that temperature increase of 8.5-11.4° C at 5 cm depth reduced the population of *Phytophthora* sp. from 38.2 to 2.0 cfu in a *Citrus* nursery (Gade & Giri 2005).

Generally most of plant pathogens and pests are mesophillic and unable to survive for long periods at temperature above 37° C. The heat sensitivity of these organisms is related to an upper limit in the fluidity of cell membranes, which lose their ability to function at high temperatures. Other causes of death of organisms at high temperature are the sustained inactivation of enzyme systems, especially respiratory ones (De Vay et al. 1990). Soil solarization completely eliminates pathogenic fungi but population of beneficial organisms such as *Bacillus* sp. and *Pseudomonas* sp. were left out in the solarized soil (Ristaino et al. 1991). After solar heating, the population of these beneficial fungi and bacteria multiply very fast, due to non-competition with pathogenic microbial flora, which are eliminated (Stapleton 1996).

In our study, the population of soil fungi and root *Aspergillus, Penicillium, Fusarium, Rhizopus* and nematodes were completely eradicated in 0-5 cm soil depth. Some population of beneficial bacteria and fungi like *Trichoderma*, *Aspergillus* and AM fungi left after solar heating (Table 2-3) confirms the previous reports. Pandey & Pandey (2004) also reported decrease in damping off tomato and chili from 32.4% to 95.6% due to solarization by reducing soil pathogens.

Occurrence of fruit bodies of ectomycorrhizal and saprophytic mushrooms (Table 4) on treated seedbeds may be due to change of

physical and chemical properties of the soil (Chen & Katan 1980). Non-competition of mushroom mycelia with common soil saprophytic fungi may be one of the reasons for spread of mycelium of fungi in the soil, up to thresh hold level to develop their fruiting bodies. Elimination of pathogenic microbes and nematodes, release of nutrients in soil may be correlated for enhanced growth in height and collar diameter of seedlings in solar heated seedbeds (Table 5). Solar heating decreased pathogenic and ectomycorrhizal inocula potential and increased soil nitrate has been reported (Salerno et al. 2000). The method has been applied to control root pathogens along with management of ectomycorrhizal fungi for production of seedlings of forest trees (Annesi & Motta 2007, Perrin et al. 1998, Pinkerton et al. 2002, Salerno et al. 2000, Soulas et al. 1997).

Conclusion

Solarization of nursery soil kills harmful microorganisms and induces appearance of fruit bodies of mushrooms, which are beneficial for growth and development of seedlings of tropical forest trees. In the present study, it is for the first time noticed that fruit bodies of mushrooms, which generally form ectomycorrhizae with suitable hosts, only appeared in solarized seedbeds and enhanced growth of seedlings of tropical tree species.

Acknowledgement

We wish to thank Dr. A.K. Mandal, Tropical Forest Research Institute, Jabalpur, for providing necessary facilities.

References

Afek U., Menge J.A., Johnson E. L. V., 1991. Interaction among mycorrhizzae, soil solarization, metalaxyl, and plants in the field. Plant Disease 75: 665-672.

Annesi T., Motta E., 2007. *Hebeloma* inoculation on Norway spruce seedlings in solarized and infested soil. European Journal of Forest Pathology 28(3): 159-166.

Chellemi D. O., Olson S. M., Mitchell D. J., 1994. Effects of soil solarization and fumigation on survival of soilborne pathogens of tomato in northern Florida. Plant Disease 78: 1167-1172.

Chen Y., Katan J., 1980. Effect of solar heating of soil by transparent polyethylene mulching on their chemical properties. Soil Science 130: 271-277.

De Vay, J. E. Stapleton, J. J., C. L. Elmore, 1990. Soil Solarization. Food and Agricultural Organization, United Nations. FAO Report #109. Rome, Italy.

Freeman S., Sztejnberg A. S. E., Katan J., 1990. Long-term effect of soil solarization for the control of *Rosellinia necatrix* in apple. Crop Protection 9: 312-316.

Gade R. M., Giri G. K., 2005. Integration of soil solarization and fungicides for management of *Phytophthora* sp. in citrus nursery. Indian Phytopathology. 58: 114-116.

Hartz T., De Vay J., Elmore C., 1993. Solarization is an effective soil disinfestation technique for strawberry production. HortScience 28: 104-106.

Joshi K. C., Jamaluddin, 2007. Handbook on diseases, insect pests and their control measures in forest nurseries / plantations. Published by Tropical Forest Research Institute (ICFRE), Jabalpur, India, 58p.

Katan J., 1981. Solar heating (solarization) of soil for control of soilborne pests. Annual Reviews of Phytopathology 19: 211-236.

Katan J., 1987. Soil Solarization. In Chet I. (ed.): Innovative Approaches to Plant Disease Control. John Wiley & Sons Inc., pp. 77-105.

Katan J., Greenberger A., Alon H., Greenstein A., 1976. Solar heating by polyethylene mulching for the control of diseases caused by soil-borne pathogens. Phytopathology 66: 683-688.

Nair S. K., Peethambaran C. K., Geetha D., Nayar K., Wilson K.I., 1990. Effect of soil solarization on nodulation, infection by mycorrizal fungi and yield of cowpea. Plant Soil 125: 153-154.

Pandey K. K., Pandey P. K., 2004. Effect of soil solarization on disease management in vegetable nursery. Journal of Mycology and Plant Pathology 34: 398-401.

Perrin R., Pierre C., Bihan L. E., Soulas M. L., 1998. Evaluation of soil solarization for controlled ectomycorrhization in temperate forest nurseries. 2nd International Conference of Mycorrhiza, 5-7 July, Uppsala, Sweden.

Pinkerton J. N., Ivors K. L., Reeser P. W., Bristow P. R., Windom G. E., 2002. The use of soil solarization for the management of soil borne plant pathogens in strawberry and red raspberry production. Plant Disease 86: 645-651.

Raj H., Sharma S. D., 2008. Integration of soil solarization and chemical sterilization with benefifial microorganisms for the control of root rot and growth of nursery apple. Scientia Horticulture 119(2): 126-131.

Ristaino J. B., Perry K. B., Lumsden R. D., 1991. Effect of soil solarization and *Gliocladium virens* on Sclerotia of *Sclerotium rolfsii*, soil microbiota, and the incidence of southern blight in tomato. Phytopathology 81: 1117-1124.

Salerno M. I., Lori G. A., Giménez D. O., Giménez J. E. and Beltrano J., 2000. Use of soil solarization to improve growth of eucalyptus forest nursery seedlings in Argentina. New Forests 20(3): 235-248.

Soulas M. L., Bihan B. L., Camporota P., Jarosz C., Salerno M. I., Perrin R., 1997. Solarization in a forest nursery: effect on ectomycorrhizal soil infectivity and soil receptiveness to inoculation with *Laccaria bicolor*. Mycorrhiza 7: 95-100.

Stapleton J. J. 1996. Fumigation and solarization practice in plasticulture systems. HortTechnology 6: 189-192.

Stapleton J. J., De Vay J. E., 1982. Effect of soil solarization on population of selected soil microorganisms and growth of deciduous fruit tree seedlings. Phytopathology 72: 323-326.

Sylvia D. M., 1994. Vesicular arbuscular mycorrhizal fungi. In: Methods of soil analysis, part 2. Microbiological biochemical properties. Soil Science Society of America, Madison, Wisconsin, USA, pp. 351-378.

Warcup J. H., 1950. The soil plate method for isolation of fungi from soil, Nature, London, 166: 117-118.

Warcup J. H., 1955. On the origin of colonies of fungi developing on soil dilution plates, Transactions of the British Mycological Society 38: 298-301.