Combined effect of soil solarization and neem amendment on survival of *Macrophomina phaseolina* sclerotia and growth of soybean

R.C. Dubey[1], Harish Kumar[1], R.R. Pandey[2]

[1.] Department of Botany and Microbiology, Gurukul Kangri University, Haridwar- 249 404, India
[2.] Department of Life Sciences, Manipur University, Canchipur, Imphal -795 003, India
profrcdubey@gmail.com, pandey.rr@rediffmail.com

Abstract: Combined effect of soil solarization and neem products (leaf, bark and oil cake powders and neem oil) amendments on the survival of *Macrophomina phaseolina* sclerotia in soil was studied. Propagules of *M. phaseolina* treated with different neem products gradually decreased with increase in duration of soil solarization as compared to control. Minimum number of sclerotia/g dry soil was recorded in 1% neem oil and 10% cake powder amended soil. Maximum viable sclerotia were detected in unsolarized control soil followed by bark powder amended soil. Soil solarization effectively caused a decline in propagules of *M. phaseolina* by 20% in comparison to unsolarized control soil after 30 days. However, the effectiveness of solarization got potentiated upon addition of different neem products. Neem cake powder posed the most toxic effect in decreasing the survivability of test pathogen sclerotia. The bacterial population was higher in solarized soil as compared to unsolarized control soil. Moreover, the bacterial counts increased after addition of neem cake powder in solarized soil. The fungal population was found to be almost equal in leaf and oil cake powders amended soils. Seedling emergence (%) of soybean in solarized and nonsolarized soils was similar but the total number of infected plants in solarized soil was lesser than unsolarized control. Combined effect of solarization and cake powder amendment minimized the number of infected plants by 60% and increased the seedling growth and biomass as compared with control.
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Key words: Soil solarization, Neem products, Sclerotia survival, *Macrophomina phaseolina*, Soybean growth

1. Introduction

Soil solarization is a non-chemical method for controlling soil borne diseases and pests other than the use of plant products as pesticide (Katan, 1981). Soil borne plant pathogens of some vegetable and fruit crops have been controlled partially by pesticides. However, the use of soil fumigants is often undesirable due to unfavorable effect on animals or human beings, resulting in toxicity on plants and soil, complexity in treatment and expensive high cost. Soil solarization captures the radiant energy of sun, thereby causing physical, chemical and biological changes in the soil. Transparent polythene sheet placed on moist soil during the hot summer months increases soil temperature to a level lethal to many soil-borne plant pathogens (Dwivedi and Dubey, 1987).

Soybean (*Glycine max* L. Merr.) is one of the major oil yielding plant found not only in India but throughout the world due to the presence of high calorie protein and edible oil. *Macrophomina phaseolina* Tassi (Goid.) is one of the pathogens that cause charcoal rot of soybean resulting in great economic loss. However, neem (*Azadirachta indica* A. Juss.) has a great potential for controlling various phytopathogenic fungi due to presence of a variety of bioactive compounds (Tewari, 1992). Effectiveness of neem extracts and oil as a fungicide has been reported by several workers (Dwivedi and Dubey, 1986; Sharma and Basandrai, 1997; Lokhande et al., 1998; Dubey et al., 2009).

M. phaseolina was reported to cause significant inhibition in germination of soybean in tarai region of Uttarakhand along with some other phytopathogenic fungi (Uma et al., 1999). Integration of soil solarization with neem product to minimize the charcoal rot disease of soybean caused by *M. phaseolina* aroused the interest to find out an ecofriendly, non-chemical technique. In the present study an attempt has been made to evaluate the combined effect of soil solarization and amendment of different neem products on the survival of *M. phaseolina* sclerotia and growth of soybean seedlings.

2. Material and Methods

2.1. Preparation of neem powders

Fresh and healthy leaves were collected from mature neem trees, washed with distilled water, dried in shade and powdered by using a mixer/grinder (Model Maharaj, Whiteline). Bark was gently removed

from the same tree, dried in shade and powdered as above. Seed cake was collected from a local Expeller mill and powdered. Neem oil procured from the Expeller mill was used as such.

2.2. Harvesting of *M. phaseolina* sclerotia *in vitro*

The sclerotia of *M. phaseolina* were harvested by cellophane disc method (Ayanru and Green, 1974). The cellophane paper discs were cut according to the size of Petri dishes and boiled for 30 min to remove plasticizers. The cellophane discs were gently spread onto the surface of Petri dishes containing solidified Potato Dextrose Agar (PDA) medium, inoculated with an agar block (5 mm diam.) of *M. phaseolina* and incubated at 30±1 ^{o}C for 5 days. On the sixth day the cellophane paper was gently removed from the surface of medium and sclerotia were harvested and placed onto sterile filter paper to dry. Dried sclerotia were mashed with mortar and pestle and filtered by a sieve of pour size 150 µm.

2.3. Survival of *M. phaseolina* in soil amended with neem products

Soil (5.5 kg) was taken from crop field and water was added to make up 50% of moisture holding capacity. Soil was autoclaved and mixed properly with 500 mg sclerotia produced as above. 250 g of soil was kept in 13 polythene bags (300 g capacity). One bag was kept as such for control. The others were amended separately with 1%, 5% and 10% of different neem products i.e. leaf, bark and cake powders. However, the neem oil concentrations applied in the present study were ca. 0.1%, 0.5% and 1.0%. All the bags were tied with a thread, punctured for gaseous exchange and incubated for 7 days at room temperature. Thereafter, 50 mg of soil (containing fungal inocula) taken out from each bag was sprinkled over solidified water agar (2%) + rose Bengal (50 mg/l) + Streptomycin (30 mg/l) medium in sterile Petri dishes in triplicates. Plates were incubated at 30±1 °C in dark for 5 days. After incubation colony forming units (CFUs) of *M. phaseolina* appeared in each plate were counted.

2.4. Effect of solarization on survival of *M. phaseolina* sclerotia in soil treated with neem products

Solar heating of soil mixed with each neem product was carried out for 30 days following the methods described by Dwivedi and Dubey (1987) and Dubey (1992). Five experimental plots (1×1 m) were selected in an agricultural area in Haldwani (Nainital district). The plots were well ploughed, evenly leveled and made free from plant debris. Three small pits (6 cm depth) for each treatment were dug in each plot. Bark powder, leaf powder and oil cake powder (@. 200 g/ha) were properly mixed with soil separately in three plots. Sclerotia (500 mg) were transferred to 15 polythene bags (300 g capacity). The bags were tied with thread and punctured with needle to facilitate moisture, heat and gaseous exchange. One bag was put in each pit and covered with soil. Two plots served as control out of which one control plot and three treatment plots were covered with 45 µm thick transparent polythene sheets. The plots were well irrigated and surface of polythene sheet was gently pressed so that it may get attached with soil surface. The edges of sheets were buried in soil to avoid the loss of moisture, heat and volatile emanating from soil. Soil moisture was regularly maintained to about 90% of its moisture holding capacity by supplying water at 3 days intervals.

After solar treatment for 10, 20 and 30 days polythene sheets were removed and bags from a pit of each plot (control unsolarized, control solarized and three neem products-treated plots) were taken out and after sampling the pits were covered as mentioned above. All samples were powdered separately with a pestle and mortar under aseptic conditions and 50 mg of sclerotia were sprinkled over the surface of sterilized and cooled 2% water agar medium and rose Bengal and Streptomycin containing in Petri dishes. Plates were incubated at 30±1 °C for a week. On the 7th day colonies of *M. phaseolina* growing on the surface of agar plates were counted and population of fungal propagules was determined on colony count basis.

2.5. Effect of neem products on microbial community of soil

Soil samples (100 g) were separately mixed with 5 g of neem products i.e. leaf, bark, cake powders and the oil (0.5 ml). The soil samples were kept in sterile polythene bags, tied with thread, punctured with needle and incubated for 7 days at room temperature. Microbial community of soil was estimated by dilution plate method. Soil fungi were isolated from 10^{-3} dilution and incubated at 25±1 ^{o}C for about a week, whereas bacteria were isolated from 10^{-5} dilution and incubated for 4 days at 30±1 °C. The colony forming units (CFUs) of fungi and bacteria g^{-1} dry soil were calculated as follows: CFUs = (Average number of colonies appeared in culture plate × 100) × Dilution factor/Weight of the oven dry soil.

2.6. Effect of soil solarization and neem products amendments on growth of soybean seedlings

After completion of solarization process soybean seeds (variety PK 416) were sown in all the five plots (control unsolarized, control solarized, leaf powder, bark powder and cake powder amended plots). After

10 days of sowing the numbers of seeds germinated were counted and seedling emergence (%) was observed using the following formula: Seedling emergence (%) = (total number of seeds germinated)/ total number of seeds sown × 100

After 30 days of sowing the soybean seedlings were removed carefully from each plot and the roots were washed gently in running tap water to remove the adhered soil particles. Healthy and diseased seedlings were categorized into two categories (infected and uninfected) on the basis of visual observation using 10 x ocular lens. Root and shoot length of each seedling and the root/shoot biomass were measured on oven dry basis at 85 °C for 24 h.

3. Results

3.1. Survival of *M. phaseolina* sclerotia in neem products amended soil

Minimum number of viable sclerotia g^{-1} dry soil was detected in 1% neem oil and 10% neem cake powder amended soil (Table 1). Maximum viable sclerotia (560 sclerotia g^{-1} dry soil) were detected in control soil, followed by 1% bark powder-amended soil (500 sclerotia g^{-1} dry soil). Neem oil was most effective in controlling the sclerotial viability, while the bark powder was the least effective.

3.2. Effect of solarization on sclerotial survival of *M. phaseolina* in neem products amended soils

Initial population of *M. phaseolina* was 440 sclerotia g^{-1} dry soil, which gradually decreased with increase in duration of solarization. Increase in temperature drastically caused a decline in population of sclerotia from 300 (unsolarized control) to 240 (solarized control) (Table 1). Further decline in number of sclerotia was potentiated after neem products amendment in soil. Only 60 sclerotia g^{-1} dry soil was survived in cake-amended soil, whereas control soil revealed 300 sclerotia g^{-1} dry soil. The cake powder was found to be the most effective in minimizing the sclerotia from 240 (control) to 60 (cake powder-amended soil) followed by leaf powder-amended soil (80 sclerotia g^{-1} soil) after the completion of solarization period (Table 1).

Table 1: Viable number of *M. phaseolina* sclerotia in solarized and unsolarized soils amended with different neem products.

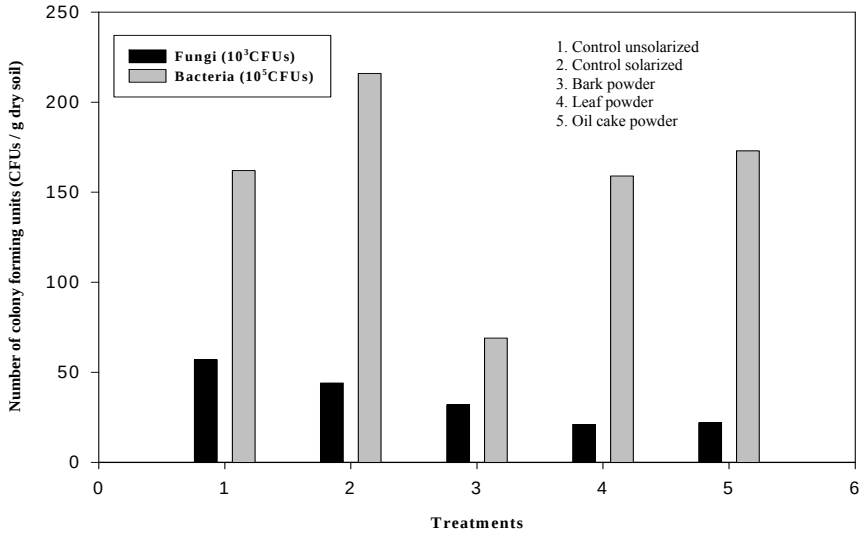
Treatment with neem products	No. of viable sclerotia g^{-1} dry soil[!]						
	Survival in lab conditions				Solarization of field		
	Concentration of neem products (%)				Duration of solarization (days)[+]		
	No.	1%	5%	10%	10	20	30
Control unsolarized	560	-	-	-	380	360	300
Control solarized	-	-	-	-	340	300	240
Bark powder	-	500	400	420	340	280	260
Leaf powder	-	320	240	160	240	140	80
Oil cake powder	-	400	340	140	200	160	60
Oil*	-	300	260	140	-	-	-

*****Concentration of oil was 0.1%, 0.5% and 1.0% instead of 1%, 5% and 10 %.
[!] Initial population of *M. phaseolina* was 440 g^{-1} dry soil ±1.
[+] Temperature recording on sampling dates:
10 days – air, 30 °C; unsolarized soil, 33 °C; solarized 39 °C
20 days – air, 29 °C; unsolarized soil, 30 °C; solarized 37 °C
30 days – air, 30 °C; unsolarized soil, 35 °C; solarized 41 °C

3.3. Effect of solarization on fungal and bacterial populations in neem products amended soils

Changes in bacterial and fungal population were measured after 30 days of solarization which revealed that the addition of neem products increased the bacterial population in solarized soils. The most abundant CFUs (173×10^{-5} g^{-1} dry soil) of bacteria were found in cake powder amended solarized soil (Fig 1). The maximum number of fungi was observed in unsolarized control (57×10^{-3} CFUs g^{-1} dry soil) followed by solarized control (44×10^{-3} CFUs g^{-1} dry soil) and bark powder amended soil (32×10^{-3} CFUs g^{-1} dry soil). The number of fungi in leaf and oil cake amended soil was almost equal.

Figure 1: Effect of different neem products on population of soil fungi and bacteria after soil solarization.



3.4. Effect of solarization and neem products amendments on growth of soybean seedlings and disease incidence

Though the emergence of seeding was equal in unsolarized and solarized control, yet disease incidence was higher in unsolarized control (85%) followed by solarized control (70%). The highest number of seed germination was observed in cake powder amended soil followed by leaf powder amended ones (Table 2). Addition of bark powder resulted in poor seedling emergence (52%) and higher disease incidence (61%) as compared to leaf and cake amendments whereas, 70-85% disease incidences were recorded in control solarized and unsolarized plots. Most of the seedlings were either disease-free or showed lesser disease incidence (1-30%). Cake powder was found to be the most effective where only 34% seedlings were infected as compared to leaf powder amended soil (43%) (Table 2). Soybean seedlings showed better growth in solarized than unsolarized soils. The maximum plant biomass yield was recorded in oil cake powder-amended soil followed by leaf powder amended ones (Table 2).

Table 2: Effect of soil solarization and neem amendments on emergence and growth of soybean seedlings.

Treatment	Growth of soybean seedlings				
	Seedling emergence (%)	Plants infected (%)	Root length (cm)	Shoot length (cm)	Dry weight (Plant biomass) (g)
Control soil					
Unsolarized	72	85	10.7	19.2	0.72
Solarized	72	70	11.0	21.3	0.92
Solarized amended soil					
Bark powder	52	61	13.0	23.4	0.95
Leaf powder	64	43	20.2	27.3	1.13
Oil cake powder	68	34	26.8	38.0	1.27

Values are the mean of 15 plants; Number of soybean seeds sown in 1×1 m^2 plots was 25.

4. Discussion

During the course of present study the field trial received huge rain fall i.e. 5 times between 2-10 days, 4 times between 10-20 days and ones between 20-30 days of solarization. The maximum temperature of mulched soil was recorded to be 41°C at 6 cm depth. Dubey (1992) has earlier reported the temperature range of 50-55.5 ^{o}C upto 6 cm depth in solarized plots amended with fungicides. Interestingly the effectiveness of solarization got potentiated upon addition of different neem products like powders of leaves, bark and seed cake. *M. phaseolina* is a high temperature loving fungus. Mihail and Alcorn (1982) have reported the thermal death range of the test fungus between 50-55 °C. However, it can tolerate temperature up to 50-52 °C for about 120 h (Paharia and Sahai, 1970). Beyond 50 °C the fungal sclerotia become inactivated within 24 h, but they are not killed (Bega and Smith, 1962). So it is possible for *M. phaseolina* to tolerate this temperature range even after a slight inactivation of some of sclerotial cells by thermal killing, because all the cells of a sclerotium function as independent unit (Wyllie and Brown, 1970). Besides thermal killing of soil-borne pathogens, plastic mulching operates its function by restoring the soil volatiles and increasing the population of antagonistic microorganisms after the completion of solarization.

Our findings revealed that the total numbers of bacterial CFUs were increased in solarized control soil, whereas the fungal counts were decreased as compared to unsolarized control. The reason for higher bacterial population in solarized soil than the unsolarized ones may be explained as perpetuation and proliferation of thermotolerant bacteria surviving after solar heating of soils. Neem products consist of toxic chemical constituents such as nimolicinol, azadirachtin, azadirachtol, nimlinone, nimbocinol, nimocin etc. with varying amounts in different plant parts (Tewari, 1992). The neem products are also subjected to microbial decomposition after their amendments in the soil. The combined effect of decomposition of neem products for 30 days in soil along with solarization process might have caused the difference in soil microbial population. Moreover, soil amendments with farmyard manure and nitrogen have been reported to increase the effectiveness of solarization by reducing the population of *M. phaseolina* (Lodha, 1995). Gaur et al. (1997) also reported that the population of *Pythium ultimum* causing damping off of linseed was decreased to 10.64 fold log cfu/g soil (90.6 %) after 30 days of soil solarization as compared to 1.16 fold reduction (14 %) in nonsolarized plots.

Though survival of *M. phaseolina* sclerotia declined to a greater extent after 30 days of solarization in the present study, a considerable number of propagules were detected in soil. It is well known that magnitude of temperature, light intensity, soil texture, soil moisture and the soil depth also play important role on the survival of microorganisms (Katan, 1981). It may be attributed that soil texture and loss of volatile fungistatic substances after lifting the polyethylene sheet during the time of sampling and watering may be the factors involved in helping sclerotial viability. The results of present study are in conformity with those of Dwivedi and Dubey (1987), Lodha and Solanki (1992), Dubey (1992) and Lodha (1995).

Increased plant biomass due to amendment of neem products may be attributed to the inhibitory volatiles emanating from decomposing powders of neem plant parts in solarized soil. These gases would have allowed changes in microbial population with increase in thermotolerant antagonistic microbes, which in turn would have caused inhibition in disease incidence caused by *M. phaseolina* and enhanced plant growth. In the present study, reduction in population of *M. phaseolina* might be the result of the triple actions of neem amendments, antagonistic activity of thermophillic microorganisms and the thermal inactivation of *M. phaseolina* sclerotia. Reduction in coconut disease caused by *Theiloviopsis paradoxa* and *Gonoderma lucidum* and the improvement in yield with application of 5 kg neem cake/palm have also been reported (Rao et al., 1992; Bhaskaran, 1993).

In conclusion our finding suggests that integrated effect of soil solarization and amendments of neem products might have played combined role in reducing the survival of *M. phaseolina* sclerotia and also the disease incidence of charcoal rot in soybean seedlings. Such disease control measure can also be efficiently used in the regions where field soil solarization is not feasible for longer duration.

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Correspondence to:

Prof. R.C. Dubey
Department of Botany and Microbiology
Gurukul Kangri University
Haridwar- 249404
Uttarakhand, India
Phone: +91-1334-246767
Fax: +91-1334-246767

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