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Soil Solarization: A Safe, Affective and Practicable Technique for the Control of Soil Born Fungi and Nematodes

¹Nafees Bacha, ¹Najma Ayub, ²Yasmin Ahmad, ³Muhammad Abbas and ⁴Abdul Rafi ¹Department of Microbiology Quaid-i-Azam University Islamabad, Pakistan ²National Agricultural Research Center CDRP Islamabad, Pakistan ³Pakistan Museum of Natural History Islamabad, Pakistan ⁴NWFP, Agricultural University Peshawar, Pakistan

Abstract: A technique i.e., Soil Solarization and Amendments (neem, chicken farmyard manure, farmyard manure and biokhad viz synthetic bio fertilizer), towards the natural cropping system has been evaluated for its effectiveness and practicability at the National Agricultural Research Center Islamabad Pakistan. Soil solarization and amendments were analyzed as a control measure against soil born fungi and nematodes. Eight weeks of solarization resulted in about 11°C increase in the soil temperature. This increase in soil temperature caused a reduction of about 70 to 80% in the fungal population and about 99% in nematode population at various depths. Neem and Biokhad amendments were proved synergistic for solarization and also improved the properties of soil in the benefit of crop plants. Fusarium sp., Macrophomina phyaseolina and Verticillium sp. of fungi and Tylenchus sp., Haplolaimus sp., Xiphenema sp. and almost all of the parasitic nematodes were significantly (p≤0.01) controlled. It was found that even after 40 days the solarized plots contain significantly less number of fungi and nematodes as compared to the nonsolarized plots, which confirmed the durability of this process.

Key words: Soil solarization, polyethylene sheath, amendments, fungal control, nematode control

INTRODUCTION

More than half of the losses caused in our valuable crop plants are due to variety of pests. These pests either directly damaging the crops or making it susceptible to the attack of a-biotic factors. From the day the enemy pests for the first time attack our economic crops, man started thinking and searching for the best and cheapest control technique that can best save these crops. For this purpose different physical, chemical and biological techniques have been developed and adopted to control the population of these pests. Most of these techniques are either costly, hard in bringing into practice, ineffective or have strong residual effect on human and animal health. Beside these the chemical control measures would be unacceptable to the environmentalists. The biological control measure on the other hand would take a considerable length of time to establish itself. So the development of a technique which is safe, effective, cheaper and easy in bringing into practice has proved a challenging job for the plant protectionists.

Recently a new technology Solarization has been introduced and is used in agriculture to reduce the use of pesticides and to control pathogenic fungi, bacteria,

nematodes and other microorganisms and also to kill weeds before and after planting of crops (Ahmad *et al.*, 1996; Abedl-Rahim *et al.*, 1988; Bettiol *et al.*, 1996; Bhaskar *et al.*, 1998). Solarization is a simple non-chemical technique that capture radiant heat energy from the sun, which increased the soil temperature depending on temperature, humidity, radiation, wind velocity and other soil characteristics (Ganguly *et al.*, 1996; Defilippi *et al.*, 1998) and caused physical, chemical and biological changes specially in the top 10 cm soil (Ahmad *et al.*, 1996; Abedl-Rahim *et al.*, 1988).

The process has the capability to increase soil temperature at various depths up to 15°C (Defilippi et al., 1998; Chandrakumar et al., 2002), this range of temperature is enough to kill most of the soil pathogen and weeds along with their dormant bodies and seeds (Chandrakumar et al., 2002; Ganguly et al., 1996). The effective of solarization depends on its duration (Ahmad et al., 1996) and the duration depends on the light intensity and day length (Ahmad et al., 1996; Chandrakumar et al., 2002). Solarization is most effective when done in June and July, however depending on the geographical location it can be effective in May, August and September (Ahmad et al., 1996).

Solarization were used alone and in combination with other treatments like Molasses Glutamic Fermentation Residue (MGFR) (Bettiol et al., 1996) Farmyard manure and mahua [Madhuca longifolia] cake (Anju and Gaur 1998) for a quick improvement of soil physical and chemical properties in the benefit of crops (Patel and Patel, 1998; Nasr et al., 1997). Since soil solarization reduced the number of fungi, bacteria, nematodes, insects and weeds, it often results in increased plant growth response, yield and fruit. Soil solarization can be used alone and in combination with various treatments even in the absence of known pathogen (Sadik et al., 1994). Soil solarization was found compatible with other physical, chemical and biological pests control measures (Ahmad et al., 1996). Macrophomina phyaseolina was best controlled (up to 100%) in the tested corn cultivar, planted in solarized plots along with stem borer, termites, mites, cut worm and fungal pathogen. i.e., F. moniliforme and F. gramminearum (Ahmad et al., 1996). While comparing to other fungal, nematode and weed control measures solarization were found very effective and economical (Montealegre et al., 1997; Mani et al., 1993).

In the present study we focused on the confirmation of soil solarization as a non-chemical, environmental friendly, stable and technically feasible control technique against fast spreading pests. To find the cost to benefit ratio of the technique in comparison to other chemical control measure was also a goal of the study.

MATERIALS AND METHODS

Conduction of trails: Field experiments were conducted in soil naturally infested with pest and pathogens at National Agriculture research Center (NARC) Islamabad Pakistan. Samples were collected from different soil depths (0-10, 10-20 and 20-30 cm) with a standard 4 cm diameter core auger to examine nutrients (part of an other experiment) before soil Solarization.

Application of treatments: Soil Solarization was used alone and in combination with different amendments i.e. Neem, Chicken farmyard manure, Farmyard manure and Biokhad to control different pests and to increase the productivity as well as quality of the tomatoes and chilies. The experimental material were kindly provided by the NARC Islamabad Pakistan. All the plots were leveled and become free of debris and large clods and then irrigation were applied before solarization, then the mulched treatments were covered with transparent polyethylene film (0.04 mm thick) to trap the solar heat in the soil. Before mulching temperature probes were inserted into the soil of all treatments and at all the selected depths. Soil was

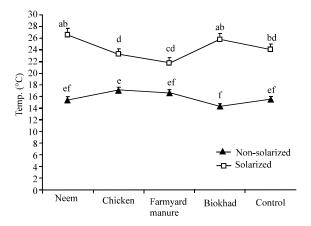


Fig. 1: Mean soil temperature (in°C) at various treatments both in solarized and in non-solarized plots. The results were statistically analyzed by using Analysis of Variance (ANOVA) for split plot design and Least Significant Difference (LSD) test at α level 0.01. Results with the same letter are insignificantly different, while results with different letters are significantly different

kept moist throughout the solarization process to increase thermal sensitivity of any resting form of pathogen and to improve heat conductivity. The polyethylene sheets were removed in the 8th week of solarization, one day before transplantation.

Studies of soil micro-flora in different treatments: Soil samples were collected from different treatment and different depths from both solarized and non solarized plots. Suspension (10⁻¹ dilution) of each sample was prepared by taking 1g in 9 mL diluents (sterile distilled water) in sterile tube, caped tightly and shacked for 30 min (Fig. 1). Then in the same manner the second (10⁻²) and third (10⁻³) Dilution were prepared. The final dilution (10⁻³) plated on the Potato Dextrose Agar (PDA) media plates with the help of micro pipette and spread with help of a sterilized glass rod and then were incubated at 25°C for 48-72 h. The number of fungal colonies were counted and then the number of fungal colony forming units (cfu) in 1g soil were calculated.

Different species of fungi at various depths and in different treatments were identified by using standard microscopic techniques.

Nematodes evaluation: For nematodes extraction and evaluation simple sieving and filtration technique has been used. Simple tissue paper is used for the filtration of nematodes. One gram soil from the bulked soil sample were carefully folded in a double layered tissue

paper. The folded sample is then placed in a 170 mesh sieves placed in nematodes collecting bath. Ten milliliter of water on then carefully added to the bath, so that the soil tissue paper is merged in the water without damaging the tissue paper. The whole arrangement is then placed for 24 h at room temperature. After this time the sieves were carefully removed from the baths, the number and different species of nematodes were then observed in 20 mL suspension.

Statistical analysis of data: Two ways Analysis of Variance (ANOVA) for split plot design and Least Significant Difference (LSD) tests were applied to analyze the results for the effectiveness of solarization, amendments and their interaction at various depths and different intervals. The results were tested under α value 0.01 and some cases 0.05

RESULTS

Increase in soil temperature during solarization: Solarization continuously and gradually increased the soil temperature at the solarized plots. Temperature at the nonsolarized plots were in the range of 14 to 17°C. All the treatments were observed for any possible effect over the soil temperature. It was observed that small variations were there in soil temperature at various treatments but

the overall effect of different amendments were negligible (Fig. 1). The soil temperature at the biokhad treatment were significantly low (14.3°C) as compared to the chicken farmyard manure (17°C) at the non solarized plot, but the same treatments were when observed at the solarized plot, it was found that chicken farmyard manure were with the second lowest (23.2°C) and biokhad treatment were the second highest. The lowest and highest temperature recorded at the solarized plots were 21.7°C (farmyard manure) and 26.5°C (neem treatment). When the temperature differences were statistically analyzed, it was found that solarization has significantly increased the soil temperature at all the treatments i.e., probability value (p≤0.01) (alphabets in Fig. 1 shows significance level). The individual effect of different treatments were insignificant in increasing the soil temperature p≥0.05 at the nonsolarized plots. The soil temperature at the solarized plots treated with neem and biokhad were significantly higher than that of chicken farmyard manure and farmyard manure p≤0.01 (Fig. 1). The control treatment was also significantly effective in increasing soil temperature at the solarized plots. The minimum and maximum soil temperature analysis at the solarized and non solarized plots revealed that solarization caused a shift of 5.1 to 11.4°C in soil temperature (compare solarized and nonsolarized lines in Fig. 1). This shift in temperature was enough to kill most of the soil pests' even spores and dormant bodies.

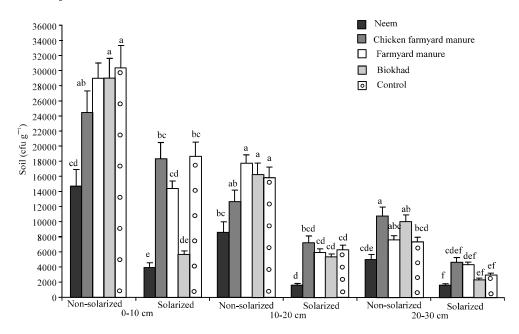


Fig. 2: Mean number of fungal colony forming units (cfu) in 1 gm soil at 0-10, 10-20 and 20-30 cm in solarized and non-solarized plots. The results were statistically analyzed by using ANOVA for split plot design and LSD test at α level 0.01. Results of each depth were calculated and analyzed independently. Results with the same letter are insignificantly different, while results with different letters are significantly different

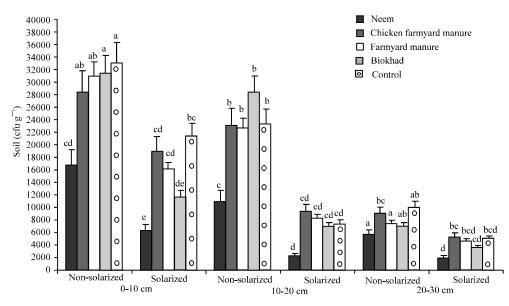


Fig. 3: Mean number of fungal colony forming units (cfu) in 1 gm of soil from three different depths 40 days after solarization. ANOVA for split plot design and LSD test were applied at α level 0.01 at each depth independently. Results with same letters are insignificantly different and those with different letters are significantly different

Fungal population soon after solarization: The soils taken from depth of 0-10, 10-20 and 20-30 cm were analyzed for fungal population soon after solarization. The culture media were inoculated with soil suspension. The fungal colony forming units (cfu) in one gram soil from all the three depths and both solarized and nonsolarized plots were calculated. The fungal cfu at the top 0-10 cm depth at the nonsolarized plots were the highest and ranged from 14000 to 32000. The neem treatment even in the absence of solarization was found effective in reducing fungal population (compare different treatments in non solarized 0-10 cm in Fig. 2). Statistical analysis of the results showed that most of the fungal species were significantly reduced (p≤0.01) in the plots solarized and amended for several weeks. Although solarization were enough effective in controlling most of the soil fungi at all the depths (control treatments Fig. 2) but their effectiveness changed with the type of treatment and depth. Neem and biokhad treatment were very effective along with solarization and has significantly i.e. p≤0.01, reduced fungal population (Fig. 2). With depth the number of fungi reduced and the smallest count of fungi were found at the depth of 20-30 cm (Fig. 1).

Mean number of fungal colonies forming units per gram of soil at different depths were very high even with the application of different amendments in the non-solarized soil i.e., 5000 (20-30 cm) to 30,000 (0-10 cm) while the cfu g⁻¹ in the solarized plots were very low i.e. 1500 (20-30 cm) to 18,500 (0-10 cm), (Fig. 2). The combined effect of some of the amendments like chicken farmyard

manure and farmyard manure did not control fungi significantly $p \ge 0.01$ (Fig. 2).

The cultural observation of the soil obtained from the solarized and non solarized plots at different depths revealed that *Macrophomina phyaseolina* were present in all the non solarized plots along with the other fungal species (Table 1). Observation revealed that the solarized plots were barren of *Macrophomina phyaseolina*. Solarization failed to fully control *Aspergillus flavus*, *Emereicella* sp. and *Penicillium* sp. (Table 1).

Fungal population 40 days after solarization: Soils from both the solarized and non solarized plots were again analyzed to conform the stability of the solarization process. Observation revealed that although with time the population of fungi flourished to grow but the effect of solarization was still observable and the fungal population at the solarized plots at all depth were significantly low i.e., p≤0.09 (Fig. 3). At the nonsolarized plots most of the amendments were insignificant i.e. p≥0.01 over the control of fungi, although the number of fungi at the amended plots were less as compared to the control nonsolarized treatment (compare control treatment with all other treatments Fig. 3). At the solarized plots chicken farmyard manure and farmyard manure were insignificant i.e., p≥0.01 against the control of various fungal species. The highest fungal count were at a depth of 0-10 cm in control nonsolarized treatment i.e., 33000 cfu g⁻¹ soil, while the lowest count were in the neem treatment i.e., 16666 cfu g-1 soil. Solarization

Tables	1: Fungal	ilora at	different	treatments	soon after	solarızatıon

	Fungal flora				
Treatments	Solarized plots	Non-solarized plots			
Neem	Aspergillus flavus, Emereicella sp.	Aspergillus flavus, A. niger, Emereicella sp.,			
		Fusarium sp. Macrophomina phyaseolina, Penicillium sp.			
Chicken farmy ard manure	Emereicella sp. Penicillium sp. Aspergillus flavus.	Fusarium sp., Aspergillus flavus, Emereicella sp.,			
-		Macrophomina phyaseolina, Penicillium sp.,			
		Helminthosporium sp., A. niger, Verticillium sp.			
Farmyard manure	Penicillium sp. Aspergillus flavus.	Fusarium sp., Aspergillus flavus, Penicillium sp.,			
-		Helminthosporium sp., A. niger.			
Biokhad	Penicillium sp. Aspergillus flavus, Fusarium sp.	Aspergillus flavus, A. niger Emereicella sp., Fusarium sp.,			
		Macrophomina phyaseolina, Penicillium sp.			
Control	Aspergillus flavus, Emereicella sp.,	Verticillium sp., Emereicella sp., Macrophomina phyaseolina,			
	Penicillium sp., Verticillium sp.	Penicillium sp. Aspergillus flavus, A. niger			

Table 2: Fungal flora at different treatments 40 days after solarization

	Fungal flora	
Treatments	Solarized plots	Non-solarized plots
Neem	Aspergillus flavus, Emereicella sp.,	Aspergillus flavus, A. niger, Emereicella sp.,
	Penicillium sp., Helminthosporium sp.	Fusarium sp. Macrophomina phyaseolina, Penicillium sp.
Chicken farmy ard manure	Macrophomina phyaseolina, Emereicella sp.	Fusarium sp., Aspergillus flavus, Emereicella sp.,
	Penicillium sp. Aspergillus flavus.	Macrophomina phyaseolina, Penicillium sp.,
		Helminthosporium sp., A. niger, Verticillium sp.
Farmyard manure	Helminthosporium sp., A. niger. Penicillium sp.	Fusarium sp., Aspergillus flavus, Penicillium sp.,
	Aspergillus flavus, Macrophomina phyaseolina	Helminthosporium sp., A. niger.
Biokhad	A. niger, Penicillium sp. Aspergillus flavus,	Aspergillus flavus, A. niger Emereicella sp.,
	Fusarium sp.	Fusarium sp., Macrophomina phyaseolina, Penicillium sp.
Control	Aspergillus flavus, Emereicella sp., Penicillium sp.,	Verticillium sp., Emereicella sp., Macrophomina
	Verticillium sp. Helminthosporium sp., A. niger,	phyaseolina, Penicillium sp. Aspergillus flavus,
	Verticillium sp., Cladosporium sp.	A. niger, Cladosporium sp.

Table 3: Nematodes population at different treatments soon after solarization

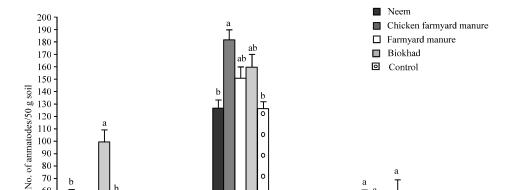
	Nematodes population			
Treatments	Solarized plots	Non-solarized plots		
Neem	<i>Rhabdits</i> sp.	(Xiphenema sp., Hoplolaimus sp., Tylenchus sp., Tylenchorhynchus sp. Helicotylenchus sp., Pratylenchus sp., Trichodorus sp.), (Diplogastroid sp., Mononchides sp., Rhabdits sp., Cephloides sp., Plecctrus sp.)		
Chicken farmy ard manure	Mononchides sp.	(Xīphenema sp., Hoplolaimus sp., Tylenchus sp., Tylenchorhynchus sp. Helicotylenchus sp., Pratylenchus sp., Trichodorus sp.), (Diplogastroid sp., Mononchides sp., Aracolamid sp., Rhabdits sp., Cephloides sp., Plecctrus sp.)		
Farmyard manure	<i>Rhabdits</i> sp.	(Xīphenema sp., Hoplolaimus sp., Tylenchus sp., Tylenchorhynchus sp. Helicotylenchus sp., Pratylenchus sp., Trichodorus sp.), (Diplogastroid sp., Mononchides sp., Rhabdits sp., Cephloides sp., Plecctrus sp.)		
Biokhad	Rhabdits sp.	(Xiphenema sp., Hoplolaimus sp., Tylenchus sp., Tylenchorhynchus sp. Helicotylenchus sp., Pratylenchus sp., Trichodorus sp.), (Diplogastroid sp., Mononchides sp., Rhabdits sp., Cephloides sp., Plecctrus sp.)		
Control	<i>Rhabdits</i> sp.	(Xīphenema sp., Hoplolaimus sp., Tylenchus sp., Tylenchorhynchus sp. Helicotylenchus sp., Pratylenchus sp., Trichodorus sp.), (Aracolamid sp., Diplogastroid sp., Mononchides sp., Rhabdits sp., Cephloides sp., Plecctrus sp.)		

reduced the highest and lowest count up to 6333 cfu g⁻¹ soil at neem treatment and 21333 cfu g⁻¹ soil in control treatment (Fig. 3). With depth the fungal population reduced and the lowest fungal count were found at a depth of 20-30 cm at both solarized plots and nonsolarized plots.

Soil analysis from all the depths and both solarized and non solarized plots revealed that the fungal species that were not present in the solarized plots (*Macrophomina physeolina* soon after solarization is now present, but in very less numbers Table 2. some new invaders i.e., *Cladosporium* sp. were also detected in the control solarized as well as non solarized plots (Table 2). Most of the fungi were detected in the 20 cm soil (Fig. 3).

Nematodes population: Soil from both solarized and non solarized plots and from three different depths (0-10, 10-20 and 20-30 cm) was examined for nematodes population. Highly significant effect of solarization on the control of nematodes were observed as p≤0.01 (Fig. 4 and Table 3).

Although the effect of some amendments like neem and biokhad were in support of our previous results and were more effective against nematodes but the internal effect of different amendments were insignificant i.e. $p \ge 0.01$, in controlling nematodes (Fig. 4). Solarizations were found equally effective at all the three depths. It was also observed that the Chicken farmyard manure treatment at the nonsolarized plots significantly increased the growth of nematodes (10-20 cm depth Fig. 4).



Pak. J. Biol. Sci., 10 (1): 57-64, 2007

Fig. 4: Mean number of nematodes in 50 soil from 0-10, 10-20 and 20-30 cm depths in solarized and non-solarized plots. ANOVA for split plot design and LSD test were independently applied at all the depths at α level 0.01. Results with the same letters are insignificantly different and with different litters are significantly different

Solarized

10-20 cm

Non-solarized

Non-solarized

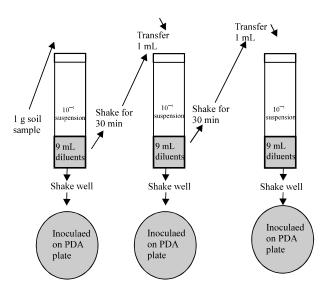


Fig. 5: Serial dilutions method for fungal analysis

Non-solarized

Solarized

0-10 cm

Mean number of nematodes in 50 g soil at a depth of 0-10 cm at the nonsolarized plots were 45-100~N/50~g of soil while solarization reduced this number to 6-14~N/50~g of soil. it was observed that the highest number of nematodes were found at 10-20~cm depth i.e., 120-175~N/50~g of soil were found in the non solarized plots while the solarized plots contain only 9-18~N/50~g soil. The numbers of nematodes at 20-30~cm in the non solarized plots were almost the same as that in the upper 0-10~cm soil (40-60~N/50~g of soil).

When the identification eassay was done as shown 5, it was surprisingly observed that all the solarized plots were almost empty of any type of parasitic specie of nematode. The different types of dominantly found parasitic and saprophytic nematode species at the non solarized plots were Xiphenema sp., Hoplolaimus sp., Tylenchus sp., Helicotylenchus sp., Tylenchorhynchus sp., Pratylenchus sp., Trichodorus sp., Mononchides sp. and Rhabditus sp. All these nematodes along with some

Solarized

20-30 cm

other were found in almost all plots regardless of treatments. When the solarized plots were observed it was found that none of the parasitic nematode specie and only *Rhabditus* sp. and *Mononchid* sp. were present in the solarized plots. This means that solarization has control significantly both the parasitic and saprophytic nematodes.

DISCUSSION

To eradicate variety of pest and to increase our crop production recently a new but improved technology Soil Solarization and Amendments has been introduced. In our experiment the soil solarization technology was used alone and in combination with various soil amendments (Neem, Chicken farmyard manure, Farmyard manure and Biokhad). The amended and non amended soil were solarized for about 8 weeks by using transparent polyethylene sheaths of 0.04 mm thickness. The thickness of polyethylene sheath affect the increase in the soil temperature during the solarization process, a 0.01 mm increase in the thickness can increase the target temperature by 2-5°C (Chandrakumar et al., 2002). The white polyethylene sheath efficiently controlled most of the pests, but polyethylene of other colors specially black color can give parallel results (Biradar et al., 1997). The duration of solarization process depends on the geographical location and can be 20 days (Chandrakumar et al., 2002), 80 days (Ahmad et al., 1996; Kumar et al., 2002a,b) or 139 days (Bettiol et al., 1996), which increased the mean temperature at 0-30 cm soil up to 11°C (Fig. 1), our results conformed some previous experiments in which several fold increase in the soil temperature i.e., 15°C, were recorded by other workers from India, Israel, Pakistan and Mexico (Bhaskar et al., 1998; Ahmad et al., 1996). Although it was solarization and not the amendments which cause the increase in temperature but the neem and biokhad treatment were useful along with solarization for a slight upward shift in temperature as compared to other treatments (Fig. 1).

It was observed that 11°C increase in temperature was enough to kill different type of pests. Solarization reduced plant pathogenic fungi 40-80% at various depths and in various amendments. Use of Neem amendment along with solarization was good companion in controlling these pathogenic fungi (Fig. 2) and in improving soil properties in the benefit of plants (data not shown). different treatments showed different effect over the control of fungi along with solarization, comparing different treatments at the same time helped us to pick the best amendment and to leave the one with weak synergistic effect. Chicken farmyard manure, farmyard

manure and control treatments were the weakest in controlling different fungal species. Our data also showed that the process was effective against these pests even 40 days after solarization. Thirty to seventy percent reduction in fungal population was observed after 40 days of the end of the solarization process. Penicillium species and Aspergillus flavus were hard to control through solarization and their population quickly start flourishing after the end of solarization (Ahmad et al., 1996; Khaleeque et al., 1999). The technique efficiently controlled Aspergillus niger, Fusarium sp. Emereicella sp., Macrophomina phaseolina, Helminthosporium sp. and Verticillium sp.

The second invaders and threat to the crop plants were the nematodes. To study whether the solarization is effective in controlling nematodes or not first soil from different depths were observed for nematode population. Both parasitic (Xiphenema sp., Tylenchorhynchus sp., Trichodorus sp., Pratylenchus sp., Tylenchus sp., Helicotylenchus sp. and Hoplolaimus sp.) and saprophytic nematodes (Aracolamid sp. and Diplogastroid species) were abundantly found in the non solarized plots Table 1. Solarization controlled about 100% of the parasitic nematodes and about 65-95% of the saprophytic nematodes. In the solarized plots only Rhabditis sp., Cephaloides sp. and Mononchide sp. i.e. saprophytic species, were isolated. Although Solarization reduced the number of these nematodes but as the solarization process finished these nematodes quickly start flourishing.

It was observed that the region between 10-20 cm were with highest number of nematode and bellow this depth the nematode population reduced (Fig. 4). Although at the non solarized plots the biokhad treatment at the upper 10 cm layer and bellow this chicken farmyard manure were found supportive to the nematodes population. But at the solarized plots the amendments were insignificantly effective over the control or over the support of nematodes (Fig. 4).

Since the Solarization reduced the number of fungi and nematodes the different amendments improve the chemistry of the soil hence it often results in increase plant growth response, Yield and Fruit quality of tomatoes and chilies (Data not available) (Abedl-Rahim *et al.*, 1988).

Because of its ease, multidimensionality, zero residual effect and cost effectiveness, solarization in the field can be the most effective technique in all the available pest control techniques. Using different amendments like biokhad and neem along with solarization can affect its efficiency and also improve the soil physical and chemical qualities in the benefit of crops.

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