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Comparative efficacy of unsolarized and solarized soil interacted with Neem cake and Carbendazim amendments effect on *Fusarium oxysporum* f.sp. *lycopersici* of tomato

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Abstract

Soil solarization is a natural, hydrothermal process of disinfecting soil from plant diseases that is accomplished through passive soil heating. Adaptation of soil solarization and integrated approaches with neem cake and Carbendazim against Fusarium oxysporum f.sp. lycopersici was undertaken in the green house conditions . A total of seven treatments, were taken up in randomized block design. Sick soil of Fusarium oxysporum f.sp. lycopersici was made @ cfu 3× 10^5/ g before solarization. After solarization FYM was applied@ 100 g / pot ,Neem cake powder @ 10g/pot and carbendazim @ 2g /10 kg soil in pot. The disease intensity (%) of Fusarium oxysporium f.sp. lycopersici at 60 days after germination was significantly reduced in T_3, T_4, T5 and T2 as compared with T1 and T0 . At 90,120,150 days, significantly reduced in T_3, T_5, T4 and T2 from T1 and T0 whereas every the treatments T0 , T1 was significant from each other . The highest reduction (%) was found in (T_3, T_2, T4, T5) as compared with (T0, T_1) .150 days after germination ,the shoot and root length , fresh and dry shoot weight significantly increased in treatment T6 (tomato plant alone) followed by T_2 (solarized soil with neem cake) as compared to control. The fresh and dry root weight significantaly increased in treatment T6 (tomato plant alone) and T_3 (solarized soil with carbendazim) as compared to control.

Key words: *Fusarium oxysporium* f.sp. *lycopersici* , Neem cake, Carbendazim, tomato plan

Introduction

Tomato (*Lycopersicon esculentum*, Mill.), is the most important tropical vegetable crop used throughout the world (Shervin *et al*, 2011).

Fusarium oxysporum is a soil borne fungal pathogen that infects plants through roots at all stages of plant growth, causes major economic losses by inducing necrosis and wilting symptoms in many crop plants (Ozbay and Newman, 2004; El- Khallal, 2007). *Fusarium oxysporum* f. sp. *lycopersici* (FOL) is economically important wilting pathogen of this crop and major limiting factor in the production of tomato. This disease cause great losses, especially on the susceptible varieties of tomato during the warm season (Agrios, 2005; Mandal *et al.*, 2009).

Control of soil born disease has been accomplished primarily by the application of chemical fungicides, long crop rotations and fumigants (Spletzer and Enyedi, 1999). Perusal of earlier literatures indicates that attention had not given for utilization integrated approaches of soil solarization followed by Neem cake amendment in the soil for the controlling of *F. oxysporum* f. sp. *lycopersici* and other plant pathogens, even if their effectiveness has been reported in reducing many diseases of tomato.

Soil solarization is considered a relatively mild heating treatment for disinfesting soils where the population of soilborne pathogens including *Fusarium oxysporum* f. sp. *lycopersici* showed significant reduction in pathogen population at the soil depths of 20 and 30 cm after seven weeks of two solarization periods (Radwan and Mohammad, 2012).

Kimaru *et al*. (2004) observed that soil amended with Neem Kernel Cake Powder suppressed the growth of *Fusarium oxysporum* f. sp. *lycopersici* .The suppressive effect might have been due to production of fungistatic substances such as azadirachtin and improved host resistance perhaps as a result of improved host nutritional status (Schafer, 1971; Khan *et al*., 1973; Maukau, 1980; Agrios, 1988). Neem seed cake contains higher levels of nitrogen, phosphorus, potassium, calcium and magnesium than those in farmyard manure or sewage sludge (Radwanski and Wickens, 1981).

Materials and methods

The experiment was conducted primarily in the field and further under pots condition in the Department of Plant Pathology, Sam Higginbottom Institute of Agriculture, Technology & Sciences, Allahabad (India) during the year 2013- 2014.

Isolation of *F. oxysporum* f. sp. *Lycopersici*:

F. oxysporum f. sp. *lycopersici* was isolated from infected tomato plant roots by tissue segment method. The infected roots were cut separately into small pieces with the help of sterile blade and surface sterilized with mercuric chloride solution (0.1%) for 20-30 seconds followed by three times rising with sterilized distilled water. The pieces were then placed on sterilized blotting paper to remove excess moisture. The surface sterilized diseased pieces were

then aseptically, transferred on czapek's dox agar medium and incubated at 28±2˚C for seven days. After incubation, white colour colonies were observed and identified on the basis of morphological and reproductive characters. The pure culture was maintained on czapek's dox agar and preserved at low temperature in refrigerator followed by the method of Raithak and Gachande , (2013).

Preparation the sick microplot of *F. oxysporum* f. sp. *Lycopersici*:

The soil was infest uniformly with the wilt pathogen, 1 L of a sand/ sorghum grains inoculum mix was incorporated into the top 45 cm of 6 microplots. The inoculum was prepared by growing a culture of *Fusarium oxysporum* f.sp. *lycopersici* in a modified liquid broth czapek's Dox (CD) (Esposito and Fletcher, 1961) on a rotary shaker with indirect fluorescent light at room temperature (22º C). After 15 days, the contents of the flask were filtered through eight layers of sterile cheesecloth. One hundred milliliters of the filtrate, which was predominantly microcondia, was mixed with 4 L of a twice-autoclaved sand / sorghum grains (4:1, v/v) mixture. The mixture was incubated 8 wk at room temperature to allow the fungus to colonize the medium extensively. To verity that *Fusarium oxysporum* f.sp. *lycopersici* had been established successfully, microplots were planted with seeds of the wilt-susceptible tomato cultivar CO - 3. After, a high percentage of wilt had occurred in all infested plots while no wilt occurred in uninfested two microplots. The remaining aboveground plant material was cut off at the soil line and removed. On 2nd May 2013, each microplot was light irrigated and covered with an ultraviolet stabilized, 30 µm clear polyethylene film in 4 microplots. The plastic was removed thrice from six microplots at 15 days of interval that is up to June 2013 (Martyn and Hartz, 1986), two microplots was kept in nonsolarized soil.

Application of Neem cake and carbendazim

The solarized and unsolarized soil was mixed with FYM @ 100 g / pot and was filled in thirty five pots. Out of thirty five, 25 pots were filled with solarized soil, All the pots were watered and kept inside the net house. Neem cake powder was amended in 10 pots capacity 10 kg soil / pot @ 10 g/ pot (kimaru *et al*,2004), whereas Carbendazim 50 % w.p was applied @ 2 kg a.i / ha on per ICAR recommendation rate. Ten seeds (CO-3) pre pot were sown, four seedlings per pot were maintained for treatments and three pots were untreated served as control.

Observation were recorded on shoot length (cm), fresh and dry shoot weight(g), root length(cm), fresh and dry root weight(g), disease intensity and reduction (%) at different days of intervals.

Measurement of disease intensity:

Disease intensity was taken after 60, 90,120 and 150 days after germination. The O-4 scale of the disease severity was classified as follows:

0: No wilt symptoms

1: Slight severity, where 25% leave become wilted and one or two leaves became yellow

2: Moderate severity, two or three leaves became yellow, 50% of leaves became wilted

3: Extensive severity, the all plant leaves became yellow, 75% of leaves become wilted and
growth is inhibited

4: Complete severity, the whole plant leaves become yellow, 100% of leaves become wilted and the plants die.

The percentage of disease intensity was determined using the formulas as given by Song *et al*. (2004).

$$\text{Disease intensity (\%)} = \frac{\sum \text{Scale} \times \text{number of plants infected}}{\text{Highest scale} \times \text{total number of plants}} \times 100$$

Reduction (%) of disease was calculated by using the following formula according to (Hend and Kahkashan *et al*., 2012).

$$\text{Reduction (\%)} = \frac{\text{Control- treatment}}{\text{Control}} \times 100$$

Details of the treatments:-

T_0- *F. oxysporum* f. sp. *Lycopersici* alone

T_1- Solarized soil + *F.o*

T_2 - Solarized soil + neem cake + *F.o*

T_3- Solarized soil + carbendazim + *F.o*

T_4- Solarized soil + neem cake + carbendazim + *F.o*

T_5- Unsolarized soil + carbendazim+ *F.o*

T_6- tomato plant alone

Results and discussion

Effect of unsolarized and solarized soil with Neem cake and Carbendazim on plant growth parameters at 150 days after germination of tomato plants.

Shoot and Root length (cm)

The result of Table 1 and fig 1 indicates the shoot length of tomato significantly increased in T_6 (124.81 cm) followed T_3 (114.60 cm), T_2 (110.20 cm) , as compared with other treatments , Among the treatments T_4 (101.80 cm) and T_5 (100.55 cm) were found non-significant from each other but

significantly increased the shoot length as compared with T_0 (50.65 cm) and T_1 (55.10 cm). The root length of tomato significantly increased in T_6 (28.65 cm), T_2 (28.60 cm) and T_3 (27.40 cm) as compared with T_4 (22.65 cm), T_5 (20.00 cm) , T_1 (11.55 cm) and T_0 (10.35 cm).

Fresh shoot and root weight (gm)

The fresh shoot weight of tomato was significantly increased in T_6 (91.35 g) as compared with T_2 (71.75 g), T_3 (60.95 g), T_4 (52.70 g), T_5 (50.40 g) from T_0 (7.50 g) and T_1 (8.65 g) . fresh root weight in treatment T_6 (6.2 g) significantly increased from T_4 (3.70 g), T_1 (0.79 g) and T_0 (0.60 g) respectevly.

Dry shoot and Root weight (gm)

Dry shoot weight was significantly increased in T_6 (61.6 g) from all the treatments as compared with the treatments T_2 (39.65 g), T_3 (34.60 g), T_4(30.75 g), T_5 (30.45 g) , T_0 (5.05 g)and T_1 (5.60 g) respectively. And dry root weight of tomato in treatments T_6 (3.30 g) significantly increased from T_5 (1.85 g) , T_1 (0.52 g) and T_0 (0.39 g) and the treatmentT_5(1.85 g) significantly increased dry root weight of tomato from (T_1 and T_0).

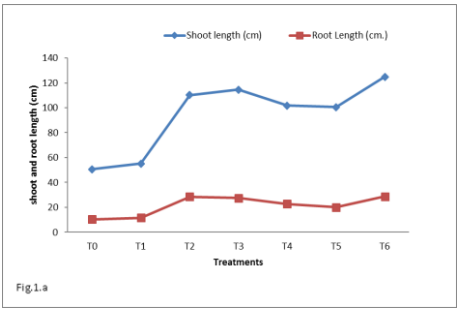
Effect of unsolarized and solarized soil with Neem cake and Carbendazim on Disease intensity (%) at different days of intervals after germination of Tomato

Observation recorded on 60, 90, 120 and 150 days after germination as shown in Table 2 and fig 2 Mean disease intensity (%) of tomato plants reveal that T3 (solarized soil with carbendazim 5.00 %), T4 (solarized soil with neem cake and carbendazim 5.00 %), T5 (non solarized soil with carbendazim 6.25 %) and T2 (solarized soil with neem cake 7.50 %) were found significant reduced the % of disease from T1 (Control solarized soil with *F.o* 23.75 %) and T0 (Control non solarized soil with *F.o* 20.00 %),T3 (solarized soil with carbendazim) 11.25, 15.00, 16.25%, T_5 (non solarized soil with carbendazim)12.50,16.25,17.25 % , T_4(solarized soil with neem cake and carbendazim) 12.50,15.00,16.75 %, T2 (solarized soil with neem cake) 15.00,20.00,21.50 % respectively was found significantly superior from T1 (Control solarized soil with *F.o*)25.00,30.85,41.40 % and T0 (Control non solarized soil with *F.o*) 36.25,49.90,65.38 % respectively .

Table 1: Effect of unsolarized and solarized soil with Neem cake and Carbendazim on plant growth parameters at 150 days after germination of tomato plants

Treatment	Shoot length (cm)	Fresh Shoot Weight (gm)	Dry Shoot Weight (gm)	Root Length (cm.)	Fresh Root Weight (gm)	Dry Root Weight (gm)
F. o alone	50.6	7.5	5.0	10.3	0.6	0.3
S.S +*F. o*	55.1	8.6	5.6	11.5	0.7	0.5

S.S + neem cake +*F. o*	110.2	71.7	39.6	28.6	4.2	2.6
S.S + carbendazim+*F. o*	114.6	60.9	34.6	27.4	4.7	3.3
S.S + neem cake + carbendazim+*F .o*	101.8	52.7	30.7	22.6	3.7	2.1
non S.S + carbendazim+*F. o*	100.5	50.4	30.4	20.0	4.4	1.8
tomato plant alone	124.8	91.3	61.6	28.6	6.2	3.3
F- test	S	S	S	S	S	S
S. Ed. (±)	8.551	15.723	8.649	2.493	0.938	0.640
C. D.(P =0.05)	18.128	33.332	18.336	5.285	1.989	1.356



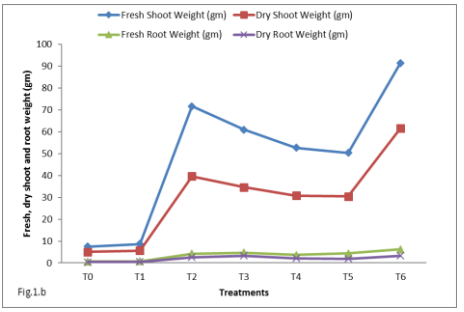
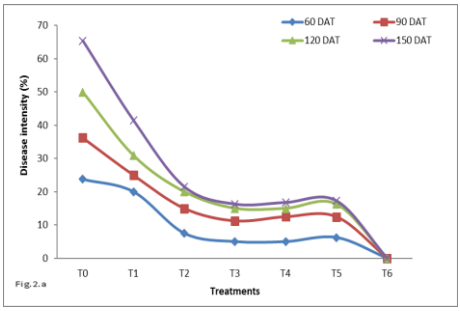


Fig 1 a and b: Effect of unsolarized and solarized soil with Neem cake and Carbendazimon plant growth parameters at 150 days after germination of tomato plants

Table 2: Effect of unsolarized and solarized soil with Neem cake and Carbendazim on disease intensity (%) at different days of intervals after germination of Tomato

Treatment	60 DAT		90 DAT		120 DAT		150 DAT	
	Disease intensity (%)	Reduction (%) over control	Disease intensity (%)	Reduction (%) over control	Disease intensity (%)	Reduction (%) over control	Disease intensity (%)	Reduction (%) over control
non S.S +*F. o*	23.7	0	36.2	0	49.9	0	65.3	0
S.S +*F. o*	20.0	15.7	25.0	31.0	30.8	38.1	41.4	36.6
S.S + neem cake +*F. o*	7.5	68.4	15.0	58.6	20.0	59.9	21.5	67.1
S.S + carbendazim+*F. o*	5.0	78.9	11.2	68.9	15.0	69.9	16.2	75.1
S.S + neem cake + carbendazim+*F. o*	5.0	78.9	12.5	65.5	15.0	69.9	16.7	74.3
non S.S + carbendazim +*F. o*	6.2	73.6	12.4	65.5	16.2	67.4	17.2	73.6
tomato plant alone	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
F- test	S		S		S		S	
S. Ed. (±)	4.910		4.321		3.819		3.131	
C. D.(P =0.05)	10.409		9.161		8.096		6.638	



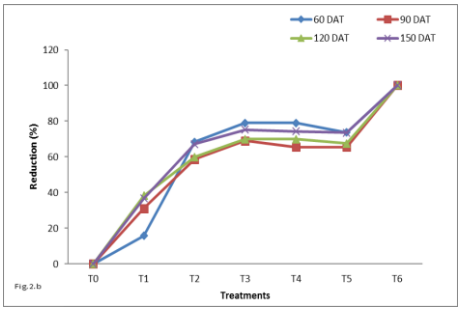


Fig 2 a and b: Effect of unsolarized and solarized soil with Neem cake and Carbendazim on disease intensity (%) at different days of intervals after germination of Tomato

Discussions

Similar finding by Kimaru *et al.* (2004) investigated the effect of Neem Kernel Cake Powder (NKCP) at 1.75, 3.5 and 7g rates on development of tomato Fusarium wilt in 1997. Performance of plants grown in NKCP amended and non-amended soils was significantly different (33.3 - 93.3%). Disease severity based on the wilt index (0.53-2.87) and length of discoloured vascular tissues (7.4cm - 25.62cm) differed significantly among treatments. Shervin *et al.* (2011) have been shown neem seed powder significantly reduced the disease severity of *Fusarium*. All the treatments significantly improved the growth of the plants as compared to untreated inoculated plants .Neem not only controlled disease but also increase in growth characters such as plant weight and length. Results suggest the possible use of neem seed powder for control of Fusarium wilt disease complex. Radwan and Mohammad, (2011) tests Soil solarization against *Fusarium oxysporum* f. sp. *lycopersici* , further they observed the longest length of time recorded for temperature above 45°C under DPE sheets were 220 hours in 2008 and 218 hours in 2009. The treatments reduced the pathogen population by 86% and the disease by 43% under the DPE treatment in 2009 and to a lesser extent by the other treatments. Increases of up to 94% in fresh plant weight and up to 60% in plant dry weight were evident under the same treatment. The treatments also increased soil organic matter, both nitrogen forms, and major cations. Martyn, and Hartz, (1986) stated that Soil solarization for either 30 or 60 days was

effective in delaying the onset of wilt symptoms as well filtered through eight layers of sterile as in reducing total disease incidence in a Fusarium-susceptible watermelon cultivar, Sugarbaby, cheesecloth. One hundred milliliters of but complete disease control was not achieved. Michael and Martin, (2010) suggested that soil solarization can be an effective tool for management of Fusarium wilt on lettuce, especially when used within an integrated program in conjunction with existing disease management tactics.

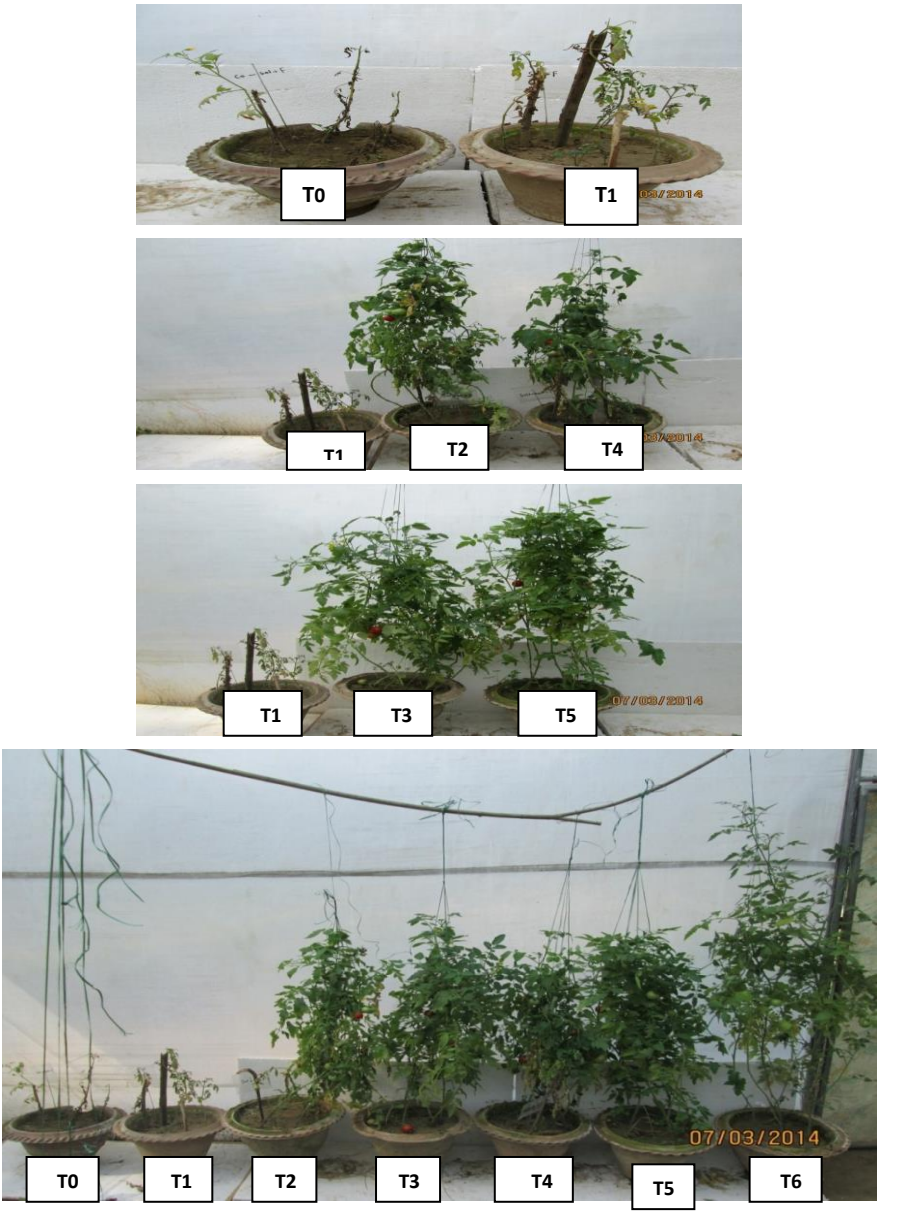


Fig 3: Effect of tretments (T0) Control unsolarized soil , (T1) Control solarized soil (T2) Neem cake , (T3) solarized soil +carbendazim , (T4) Neem cake + carbendazim ,(T5) unsolarized soil +carbendazim and (T6) tomato plant alone on tomato plants with *F. oxysporum* at 142 days after germination

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