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Effect of soil solarization, bio-agent and organic composts for the management of fusarium wilt of chickpea

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Abstract: A field experiment was conducted to compare the effect of soil solarization, *Pseudomonas fluorescens*, FYM, vermicompost and neem cake against fusarium wilt of chickpea. Soil solarization was accomplished covering by transparent polythene in summer season for 45 days (during April to May, 2012). At the time of solarization, another treatments *viz*., *Pseudomonas fluorescens* @ 2.5 kg ha^{-1}, FYM @ 10 t ha^{-1}, vermicompost @ 10 t ha^{-1} and neem cake @ 500 kg ha^{-1} were amended in the soil. After two week, seed of chickpea var. K-850 @ 40 kg ha^{-1} was shown at the space of 30 cm row to row. Results revealed that *Pseudomonas fluorescens* shows significantly minimum percentage of wilt incidence (4.40, 18.93, 31.70%) followed by Soil solarization (10.53, 23.86, 35.16%), neem cake (10.40, 28.96, 38.13%), vermicompost (17.03, 36.50, 45.76%) and FYM (19.23, 39.46, 46.96) at 30, 60 and 90 days after sowing (DAS), respectively. However, growth parameters and seed yield of chickpea were increased in the treated plots with *Pseudomonas fluorescens* followed by rest of treatments including with control.

Key words: Bio-agent, *Fusarium oxysporum* f.sp. *ciceris*, Organic composts, Soil solarization

Introduction

Chickpea (*Cicer arietinum* L.) is an important pulse crop and India ranks first in the world as per the latest report of Food & Agriculture Organization (FAO) for 2011. India is the largest producer and consumer of chickpea in the world. It was grown on about 11.9 million hectares in 2010. While in 2013-14 (second advance estimates) record production of chickpea is 9530.0 thousand tone. Chickpea is a vital source of plant derived edible protein approx. 19.3-25.4%, and is a good source of carbohydrates, minerals and trace elements also (Huisman and Van der Poel, 1994). Among the diseases of chickpea crop, vascular wilt caused by an important obligate biotroph *Fusarium oxysporum* f. sp. *ciceris* (Padwick) is considered one of the limiting factors for its low productivity. Although the disease is wide spread in the chickpea growing areas of the world, it is most prevalent in the Mediterranean Basin and the Indian subcontinent (Jalali and Chand, 1992). Fusarium wilt epidemics cause significant annual losses of chickpea yields which, account for 10 to 15% of the total yield and sometimes escalate to 100% under favourable conditions (Navas-Cortés *et al.,* 2000). In India it is 10–15%, which in years of severe epidemics may rise to 60-70% (Jalali *et al.,* 1992). The management of this disease is very difficult due to long saprophytic survival ability of the pathogen in soil. Despite the use of resistant cultivars, the occurrence and development of new pathogenic species is a continuous problem. Therefore, the application of fungicides is a normal practice; but the excessive misuse of a wide range of fungicides has resulted this to be harmful to the environment and increased the resistant pathogen populations (Ogzonen *et al.*, 2001). *F. oxysporum* f. sp. *ciceris* becomes resistant to those chemical fungicides. The integrated management strategies by using the organic materials, bioagents and cultural practices (soil solarization) should be the solution to maintain the plant and soil health as well as the environment (Siddique *et al.,* 2013). Considering above factors, the experiment was conducted to develop an integrated management practice to reduce disease by reducing the use of chemical fungicides and increase yield of chickpea.

Materials and methods

A field trial was conducted during *rabi* season, 2012-2013 at wilt seek field located at SHIATS, Allahabad. Chickpea plant showing typical wilt symptoms has grayish green chlorosis. Leaves eventually become dull yellow in color, wilt and the plant collapse and dies. This type of symptoms was supported by the literature of Singh (2009). For the purpose of isolation, uprooted the infected plants and brought in the laboratory of Plant Pathology.

Isolation of Pathogen: The roots and stem of infected plants were washed in running tap water to remove soil before isolation to avoid contamination. The roots cut into 2-3 mm small bits by the help of sterile razor blade. These bits were then surface sterilized with 0.1% mercuric chloride solution for 30-60 seconds and washed with three changes of sterilized water and put on the sterilized filter paper sheets for drying. These segments were then placed on PDA (Plate 1A and 1B) poured petri plates and were incubated at

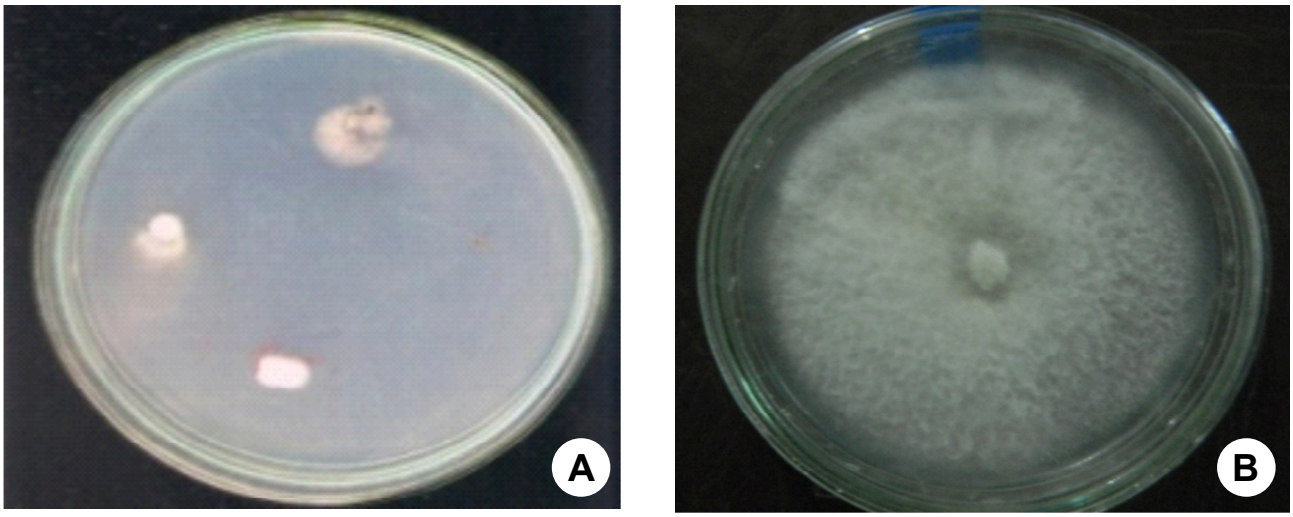

Plate-1: A. Isolation of *Fusarium oxysporum* f. sp. *ciceris* B. Pure culture of *Fusarium oxysporum* f. sp. *ciceris*

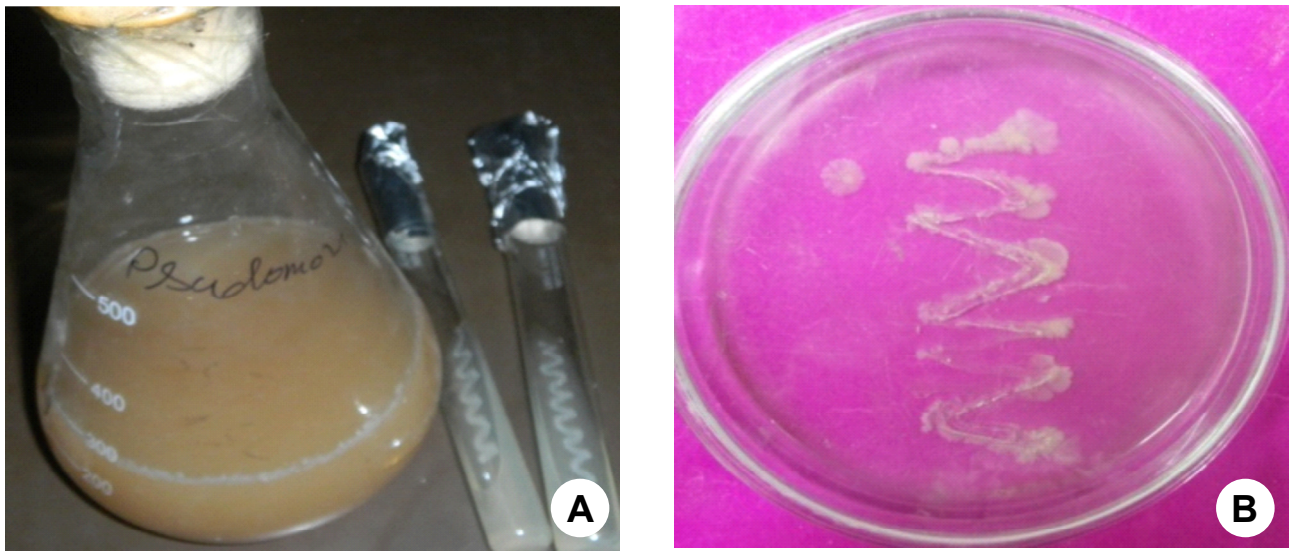

Plate-2: A and B. Pure culture of *Pseudomonas fluorescens*

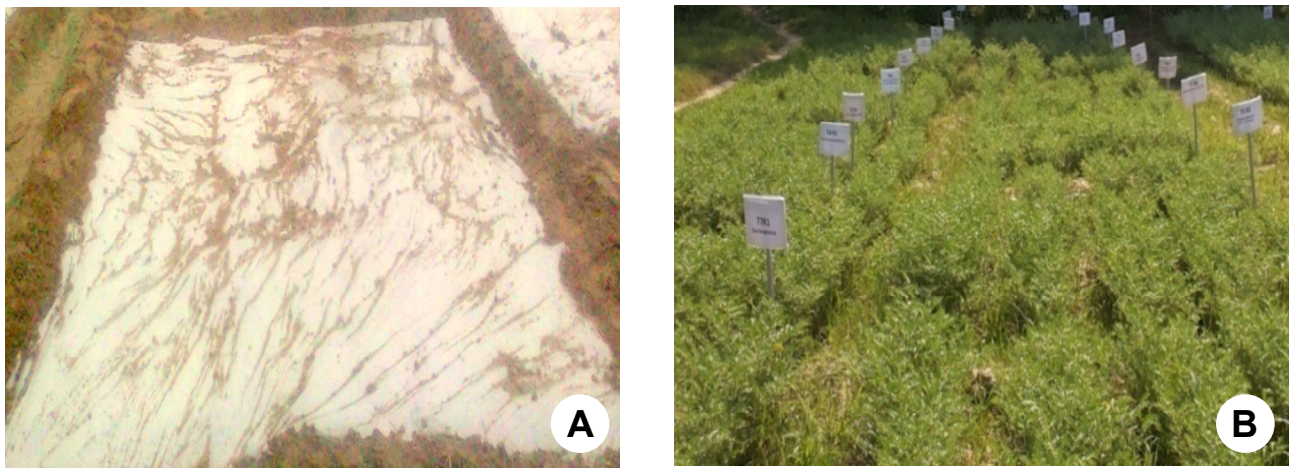

Plate-3: A. Process of soil solarization B. View of field experiment

Table-1: Effect of soil solarization, *Pseudomonas fluorescence* and organic compost on wilt disease of chickpea

Treatments	% Disease Incidence at different DAS*		
	30 DAS	60 DAS	90 DAS
T_1 (*F.o.c* .+ *P. fluorescens*)	4.40c	18.93e	31.70d
T_2 (*F.o.c* + Soil Solarization)	10.53b	23.86d	35.16cd
T_3 (*F.o.c.* + FYM)	19.23a	39.46b	46.96b
T_4 (*F.o.c.* + Vermicompost)	17.03a	36.50b	45.76b
T_5 (*F.o.c.* + Neem cake)	10.40b	28.96c	38.13c
T_0 (*F.o.c* alone)	21.66a	60.80a	81.06a
CD@5%	5.18	4.29	5.35

F.o.c. - *Fusarium oxysporium* f. sp. *ciceri* , * - Mean of three replications

25±5°C for one week. After four days, the bits were grown as a whitish colony growth and taken few parts of mycelium with the help of sterile inoculation needle on the clean slide and observed under the compound microscope. Isolation procedure was followed by the method of Aneja (2004). The isolated pathogen was identified on the basis of macro and micro conidia. After that pathogen *F. oxysporum* was purified by single spore method and was identified with the help of relevant literature of the Wollen and Reinking (1935). The mass culture of *Fusarium oxysporum* f.sp. *ciceris* was prepared on sorghum grain medium using the method of Khan *et al.* (2004).

Isolation of *Pseudomonas fluorescens* : Isolation of *Pseudomonas* spp. were made from the rhizosphere soil of various chickpea growing field of Allahabad district by serial dilution method. The rhizosphere soil was collected by gently lifting plants from the moistened soil and the loosely adhering soil with roots was collected (Johnson *et al.,* 1959). One gram of the soil was suspended in the test tube 10 ml of sterilized water and was shaken thoroughly to mix soil particles to get uniformly dispersed. Serial dilution 10^{-2}, 10^{-3}, 10^{-6} are made by pipettes measured volumes (1ml in 9ml of sterilized H_2O). Finally one ml aliquot of 10^{-6} are added to sterilized, petriplates, triplicates to which are added 15 ml. (approx.) of the sterilized cool molten (45 ^{0}C) King's B medium (King *et al.,* 1954) and maintained on king's B medium slants at 40^{0}C after growing for 2 days at 28±2^{0}C. The above bio-agents were formulated in CMC (Carboxy methyl Cellulose) with talc powder and uniform formulations @ 4×10^8cfu/g were maintained (Simon and Anamika, 2011).

Soil solarization and organic compost amendment into soil : Infected field was prepared before treatment by inoculation of mass culture of pathogen to soil. Soil solarization was done during the hottest summer month (April to May) using transparent polythene sheet (400 gauges) for 45 days, polythene sheet lade on trial field in such a way that there was no air gap. Soil was placed around the edges each polythene sheets (Plate 3A). Polythene was removed after 45 days of solarization (Cauhan *et al*., 1998). Organic amendment such as Farm Yard Manure @ 10 ton/h, Vermi compost @10 ton/h and Neem cake @ 500kg/h (Nikam *et al.,* 2007) were applied two weeks before seed sowing and irrigated as per requirement. The experiments was laid out in randomized block design with three replication plot size 2x2 m^2 was prepared, 3 plots are without any treatment was taken as control. Chickpea seeds var. K-850 @ 40 kg ha^{-1} were sown with spacing 30 cm within rows. All plots were hand weeded twice (at 3rd and 7th week after planting) (Plate 3B) (Devi *et al.,* 2013). For disease assessment the percentage disease incidence was calculated (by dividing the total number of plants infected by the disease by total number of plant observed multiplied by the 100). Plant growth parameter and yield was also calculated.

Results and Discussion

The incidence of wilt disease was reduced to some extent in all the treatments over control and statically were significant to each other. The data shows in (Table 1); revealed that wilt incidence of chickpea was significantly reduced in the treated plots with *Pseudomonas fluorescens* (4.40, 18.93, 31.70%) followed by soil solarization (10.53, 23.86, 35.16%), neem cake (10.40, 28.96, 38.13%), vermicompost (17.03, 36.50, 45.76%) and minimum disease control in FYM (19.23, 39.46, 46.96) at 30, 60 and 90 DAS, respectively. However, the treatments FYM and vermicompost amended plots were showed non-significant effect to each other. Our results are supported by Kumar *et al*. (2013); observed in field trial that seed treatment with Fluorescent pseudomonas gave significant reduction in disease incidence because its produces secondary metabolites with antibiotic activities and suppressed many soil borne diseases (Thomashow and Weller, 1996). Kumar *et al.* (2007) suggested the extracellular secretion of antifungal by *Pseudomonas fluorescens* and also suggested a significant role of secondary metabolites such as antibiotics siderophore in suppression of fungal pathogens. Zakaria and Lackword (1980) observed the suppressive ability of neem organic amendment in inhibiting growth of soil borne pathogens it to be through competition, antibiosis or due to increase of soil microbial populations. Chattopadhyay *et al.* (1999) reported that soil application of neem

Table-2: Effect of soil solarization, *Pseudomonas fluorescens* and organic compost on growth and yield of chickpea plant

Treatments	Growth parameters				Yield (t/ha)	% Yield increased over control
	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)		
T_1 (*F.o.c.* + *P. fluorescens*)	49.66a	18.53a	19.43a	8.26a	15.40a	17.84
T_2 (*F.o.c* + Soil Solarisation)	46.53b	16.50b	15.76b	6.46b	14.50a	16.80
T_3 (*F.o.c.* + FYM)	42.50c	15.26c	13.60c	5.73c	11.13c	12.89
T_4 (*F.o.c.* + Vermicompost)	45.80b	15.13c	15.33b	6.33b	10.90c	12.63
T_5 (*F.o.c.* + Neem cake)	44.10bc	16.06b	14.03c	5.43c	13.43b	15.56
T_0 (*F.o.c* alone)	29.63d	8.56d	9.26d	3.26d	8.63d	—
CD@5%	2.58	0.76	1.14	0.58	0.97	

cakes and farm yard manure lead to significant reduction of population of *Fusarium oxysporum* f. sp. *ciceri.*

The application of *Pseudomonas fluorescens* significantly increased the shoot/root length (cm) (49.66, 19.43) and shoot/root weight (g) (18.53, 8.26) as compared with other treatments. These results are supported by Burd *et al.* (2000) who reported that PGPR might enhance plant height and productivity due to synthesizing phytohormones, increasing the local availability of nutrients, facilitating the uptake of nutrients by the plants decreasing heavy metal toxicity in the plants antagonizing plant pathogens. *Pseudomonas* spp. responsible for increasing root elongation was also reported by O' Sullivan and O' Gara, (1992). Soil solarization also shows increased growth of chickpea plant. Among organic compost Vermicompost increases the shoot –root length (45.80, 15.33) and shoot- root weight (15.13, 6.33) of plant. Singh *et al.* (2010) also reported beneficial effect of Vermicompost and FYM on the growth and yield of chickpea. These finding are in conformity with the findings of Shrikrishna *et al.* (2004) in chickpea.

Yield obtained was similar to the effectiveness of treatment *i.e.* maximum in *Pseudomonas fluorescens* (15.40t/h) with percent increase (17.84%) over control (8.63t/h) followed by Soil solarisation, Neem oil cake @ 5t/h > Farm Yard Manure @ 10 t/h > Vermicompost @ 10 t/h > Control. The increase in the dry weight and yield of plants treated with *P. fluorescens* was apparently due to the plant growth promoting activity of the biofungicide Khan and Akram, (2000). This plant growth promoting effect of *P. fluorescens* may be due to the solubilisation of phosphorus in the soil Dube and Yeole, (1997). Baily and Lazarovits (2003) reported that organic amendments, manures and composts with high nitrogen contents may suppress soil borne diseases by releasing allelochemicals during microbial decomposition.

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