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Integration of soil solarization with bio-control agents for the management of stem rot of chrysanthemum

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Abstract
A study was conducted during 2016 at Nauni to find out the Effect of soil solarization with transparent polyethylene mulch (25µm thick) was recorded on soil temperature in the earthen pots and pathogenic potential of the stem rot pathogen mixed in the soil of the earthen pots. Soil solarization increased average maximum soil temperature to 45.9 °C, with an increase of 7.8 °C at 5 cm soil depth over unsolarized pots. Effect of combination of soil solarization and biocontrol agents was also observed on disease causing capability of the Rhizoctonia stem rot pathogen. *Trichoderma viride* was found most effective with no incidence of the disease and this treatment combination reduced the viability of the sclerotia by 99.2 per cent in comparison to unsolarized control.

Keywords: soil solarization, management, stem rot, chrysanthemum

Introduction
Chrysanthemum (*Dandranthema grandiflora* Tzvelev) is one among the most popular commercial cut/loose flowers of the world. Chrysanthemum is preferred for export owing to its excellent keeping quality, wide range of forms, colours and ability to withstand long distance transportation. Cut chrysanthemum and carnation contribute close to 50% of the world cut flower trade (Jawaharlal *et al.*, 2009). Among fungal diseases, Rhizoctonia stem rot (*Rhizoctonia solani*), Septoria leaf spot (*Septoria chrysanthemi*, Altenaria leaf spot (*Alternaria* spp.), Fusarium wilt (*Fusarium oxysporum* f.sp. *chrysanthemi*), rust (*Puccinia chrysanthemi*) and Powdery mildew (*Erysiphe cichoracearm*) are important. Amongst different diseases of chrysanthemum, Rhizoctonia stem rot caused by *Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris* [Frank] Donk.) is one of the most important fungal disease which mainly cause damping off, stem rot, stem girdling and root rot (Parmeter, 1970). However, the continuous use of fungicides adversely affects the soil ecosystem. On the other hand, soil solarization is an effective method for the management of stem rot and other soil-borne pathogens (Katan, 1981) [10]. Thus, the present investigation were planned with an aim to combine treatment of soil solarization with bio-control agents like *Trichoderma viride*, *Chaetomium globosum*, *Pseudomonas fluorescens*, and *Bacillus subtilis* for effective management of the stem rot of chrysanthemum.

Materials and Methods
The experiment was laid out at the experimental farm of the Department of Plant Pathology in the year 2016. Experimental design was completely randomized design with 4 replication of each treatment.

Effect of biological control agents on the viability of sclerotia of the pathogen (*R. solani*)
Effect of biological control agents on the viability of the sclerotia of the pathogen. Fifty sclerotia of the pathogen were buried in each pot in different soil layers in the root zone. All the earthen pots were watered to saturation level. Half of the pots were solarized with transparent polyethylene mulch (25µm thick) for 40 days during the months of May-June. After that polyethylene sheet was removed and then talc based formulation of *Trichoderma viride* and *Chaetomium globosum* and liquid formulation *Pseudomonas fluorescens*, and *Bacillus subtilis* were mixed up in the five sterilized pot soil infested with fifty numbers of sclerotia of the pathogen. Sclerotia were kept in these pots for two months. Out of 50 sclerotia, 20 sclerotia from solarized and unsolarized pots were retrieved after two months and their

viability was observed on the PDA. The sclerotia withdrawn were dipped in 1% Sodium hypochlorite for one minute and then sclerotia were washed thrice with sterilized distilled water in Petri plates. Sclerotia were dried by keeping them in sterilized filter papers. To observe the viability of the 20 sclerotia from each replication from each treatment were inoculated on a sterilized Petri-plate containing potato dextrose agar medium. These Petri plates were incubated at 25±1ºC temperature and observations on viability of sclerotia were taken till all sclerotia in control had germinated.

Effect of biological control agent on the potential of the pathogen to cause the disease

Effect of biological control agents on the disease causing capability of the sclerotia of the pathogen after subjecting them to the treatments of soil solarization. Biological control agents like talc based formulation of *Trichoderma viride* (20g/m^2, CFU-2×10^6) and *Chaetomium globosum* (20g/m^2) and liquid formulation of *Pseudomonas fluorescens*, and *Bacillus subtilis* (50ml/plant, CFU- 2×10^9) were mixed in the sterilized soil. Sclerotia (30 in number) were retrieved after solarization from both solarized and unsolarized pots and were mixed in each earthen pot in solarized soil added with different biological control agents. Each treatment was replicated four times with five chrysanthemum cutting raised in each earthen pot and disease incidence was recorded in each treatment.

Result and Discussion

Effect of soil solarization on soil temperature in earthen pots

Soil solarization with transparent polyethylene sheet (25 µm thick) was done for 40 days from 15th May to 23rd June during 2016 in earthen pots. Soil solarization with transparent polyethylene sheet resulted in increase in the maximum soil temperature (Table-1).

Average maximum soil temperature in the solarized soil of the pots was 45.9 °C during 2016, respectively at 5 cm depth in comparison to 38.0 °C in unsolarized pots. During 2016, soil solarization with transparent polyethylene sheet at 5 cm resulted in 7.8 °C increase in average maximum soil temperature with range of 42-49 °C during the period of solarization. However, average maximum soil temperature in the solarized soil at 15 cm depth, was 41.6 ºC during 2016, respectively in comparison to 34.6 °C in unsolarized pots.

There are no specific reports of soil solarization in pots but the principle of soil solarization remains the same. Raj *et al.* (1997) [18] reported that mulching with polyethylene resulted in 13.5 °C higher temperature at 8 cm soil depth with average maximum temperature of 49.7 °C. Negi (2009) [13] recorded an increase of 5.6 °C in average maximum soil temperature at 5cm soil depth during soil solarization with transparent polyethylene sheet in the polyhouse. Mulching with transparent polyethylene mulch resulted in 11, 8, 7 and 5 °C increase in average maximum soil temperature in comparison to non-solarized fields, at 5, 10, 20 and 30cm soil depth, respectively (Cimen *et al.* 2010) [4]. Hermanto (2012) [7] also reported that solarization with polyethylene sheet increased the mean maximum soil temperature by 10.2 °C in comparison to control. Similar type of findings have been reported from different parts of the world where soil solarization is reported to cause increase in the average maximum soil temperature (Jacobson *et al.* 1980; Katan, 1981; Chauhan *et al.*, 1988; Raj and Gupta, 1996; Raj and Upmanyu, 2006) [9, 2, 16, 17, 10].

Table 1: Effect of soil solarization with transparent polyethylene sheet (25 µm thick) on maximum soil temperature in the earthen pots

Treatments	Soil depth (cm)	Maximum soil temperature (°C) during May- June 2016	
		Average	Range
Soil covered with transparent polyethylene mulch (25 µm thick)	5	45.92	42 - 49
	15	41.60	38 - 46
Unsolarized	5	38.08	40 - 44
	15	34.69	33 - 38

Effect of combination of soil solarization and biological control agents on the viability of sclerotia of the pathogen

All the bio-control agents significantly reduced the viability of the sclerotia in the solarized and unsolarized soil of the earthen pots (Table-2). Treatment combination of *Trichoderma viride* with soil solarization was found most effective with only 0.5 per cent viability followed by 1.5 per cent viability in *Pseudomonas fluorescens* and 2.5 per cent in *Bacillus subtilis* in comparison to 69.5 per cent in unsolarized control. However, *Chaetomium globusum* was found least effective with 11.5 per cent viability of the sclerotia. In case of unsolarized soil, *Trichoderma viride* again was found effective with 61.05 per cent viability followed by *Pseudomonas fluorescens* and *Bacillus subtilis* with 62.5 and 63.5 per cent viability of sclerotia, respectively in comparison to 69.5 per cent in unsolarized control. However, *Chaetomium globusum* was found least effective with 64.5 per cent viability of the sclerotia. Soil solarization alone was also found effective with only 11.5 per cent viability of sclerotia in comparison to 69.5 per cent in unsolarized control.

Bio-control agents have been reported effective against sclerotia of different fungi as found in the present study. Santos and Dhingra (1980) reported that *Trichoderma koningii* and *Trichoderma harzianum* killed 62-100 per cent of the sclerotia of *Sclerotinia sclerotiorm* within 25 days under *in vitro* conditions. Jones and Bacon (1974) reported that 1, 3-β-glucanase is the key enzyme involved in the destruction of sclerotial wall by *Trichoderma viride*. The soil and seed treatments with *Trichoderma viride* reduced infection of sunflower by *S. sclerotiorum* and *Botrytis cinerea* in glass house and also prevented infection in the field (Sesan and Csep 1994).Sharma and Basandri (1997) [20] reported that *Trichoderma harzianum* reduced the viability of sclerotia of *Sclerotinia sclerotiorum*. Marwah *et al.* (2007) [12] and Zhang and Yang (2007) [23] reported that many strains of *Cheatomium* species inhibited the growth of bacteria and fungi through competition (for substrate and nutrients), parasitism, anti-biosis, or diverse combinations of these.

Table: 2 Effect of combination of soil solarization and biological control agents on the viability of sclerotia of the pathogen

Treatments		Per cent viability of the sclerotia after 2 months		
		Solarized	Unsolarized	Mean
Trichoderma viride		0.50 (2.03)	61.50 (51.65)	31.00 (26.84)
Pseudomonas fluorescens		1.50 (4.91)	62.50 (52.24)	32.00 (28.58)
Bacillus subtilis		2.50 (8.98)	63.50 (52.86)	33.00 (30.92)
Chaetomium globusum		9.50 (17.88)	64.50 (53.42)	37.00 (35.65)
Control		11.50 (19.67)	69.50 (56.51)	40.50 (38.09)
Mean		5.10 (10.70)	64.30 (53.34)	
CD (0.05)	Solarization	(2.12)		
	Treatment	(3.35)		
	Treatment × Solarization	(4.73)		

*Figures in parentheses are arc since transformed

Effect of combination of soil solarization and biological control agent on the potential of the pathogen to cause the disease

All the bio-control agents significantly reduced the disease incidence of stem rot in the solarized and unsolarized soil of the earthen pots in comparison to control (Table-3). Treatment combination of *Trichoderma viride* with soil solarization was found most effective with no incidence of the stem rot followed by *Pseudomonas fluorescens* and *Bacillus subtilis* with 4.1 and 4.1 per cent incidence of stem rot, respectively and both were found statistically at par. *Chaetomium globusum* was found least effective with 16.6 per cent disease incidence in comparison to 54.1 per cent in unsolarized control. In case of unsolarized soil, *Trichoderma viride* again was found most effective with 25.0 per cent disease incidence followed by *Pseudomonas fluorescens* and *Bacillus subtilis* with incidence of 33.3 and 37.5 per cent incidence of stem rot respectively in compression to 54.1 per cent in unsolarized control. *Chaetomium globusum* was found least effective with 45.8 per cent disease incidence.

Similar trends were observed for incubation period taken for appearance of the disease in the solarized and unsolarized soil of the earthen pots in comparison to control (Table-3). *Trichoderma viride* was found most effective with no symptoms of the disease. Treatment combination of soil solarization with *Pseudomonas fluorescens*, *Bacillus subtilis* and *Chaetomium globusum* were found next in efficacy as the disease took 98.0, 97.0 and 92.5 incubation days, respectively as compared to control (90.5 days). All these three treatments were statistically at par with each other. In case of unsolarized soil, *Trichoderma viride* again was found effective as 68.5 days were taken for disease to appear followed by *Pseudomonas fluorescens* and *Bacillus subtilis* with disease symptoms appearing in 67.2 and 66.7 days, respectively in comparison to control (60.5 days). Chet and Elad (1982) [3] found that application of *T. harzianum* significantly reduced the incidence of *Rhizoctonia solani* and *Sclerotium rolfsii*. Similarly, applications of *T. harzaianum* and *T. viride* are reported to be effective in controlling soil-borne diseases in comparison to non-solarized soil (Sharma, 2000) [21]. Inhibitory effect of different species of *Trichoderma* and *Bacillus* spp. have been reported by various workers against soil borne pathogens (Obiegilo, 1992; Utkhede, 1993; Pandey and Upadhyay, 2000; Ramesh and Korikanthimath, 2004; Hatvani *et al*., 2006) [14, 22, 15, 19, 6]. *Trichoderma* exert different mechanisms as antagonist for the control of plant pathogens which include mycoparasitism, competition for space and nutrients, secretion of antibiotics and fungal cell wall degrading enzymes (Kubicek *et al.*, 2001; Howell, 2003; Benitez *et al.*, 2004; Harman *et al.*, 2004) [11, 8, 1, 5]. Chandel and Sharma (2014) reported that *T. viride, T. harzianum* and *Bacillus* spp. reduced the disease incidence of carnation stem rot caused by *R. solani*. Rajendraprasad *et al.* (2017) reported that soil application of *Trichoderma harzianum* and *Pseudomonas fluorescens* reduced the disease incidence of tomato damping off caused by *Sclerotium rolfsii*.

Table: 3 Effect of combination of soil solarization and biological control agent on the potential of the pathogen to cause the disease

Treatments		Disease incidence (%)			Incubation period (Days)		
		S	US	Mean	S	US	Mean
Trichoderma viride		0.00 (0.00)	25.00 (29.66)	12.50 (14.83)	0.00	68.50	34.25
Pseudomonas fluorescens		4.17 (6.02)	33.33 (34.53)	18.75 (20.27)	98.00	67.25	82.62
Bacillus subtilis		4.18 (6.03)	37.50 (37.32)	20.83 (21.67)	97.00	66.75	81.87
Chaetomium globusum		16.66 (24.08)	45.83 (42.55)	31.25 (33.31)	92.50	61.00	76.75
Control		20.83 (26.87)	54.17 (47.41)	37.50 (37.14)	90.50	60.50	75.50
Mean		9.16 (12.60)	39.16 (38.30)		75.60	64.80	
CD (0.05)	Solarization	(5.71)			(9.79)		
	Treatment	(9.02)			(21.90)		
	Treatment × Solarization	(NS)			(30.97)		

*Figures in parentheses are arc since transformed

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