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Seed biopriming integration with soil solarization in enhancing protection against damping-off disease in solanaceous vegetable crops

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Seeds are the vital part for raising healthy nursery of solanaceous vegetable crops as they are susceptible to wide range of seed and soil borne pathogens. Nursery health is of prime importance for getting a healthy crop with higher production. Damping-off disease, a damaging factor in nursery raising of solanaceous vegetable crops appears in two stages i.e. pre and post emergence stages. Chemical based methods are most effective in managing the disease but due to the environmental concerns, there usage is discouraged. Thus, among non-chemical, environmentally sustainable method for managing the soil-borne pathogens, seed biopriming and soil solarization are of prime importance. Soil solarization causes direct thermal inactivation of soil-borne pathogens, enhances the soil microbial antagonism with specification of being accepted as potential alternative for chemical soil disinfection. Biocontrol agents like *Trichoderma*, *Pseudomonas*, *Bacillus*, *Azospirillum*, *Azotobacter*, *Rhizobium* can be used as potent agents for seed biopriming. Plant Growth Promoting Rhizobacteria (PGPRs) are potent seed biopriming agents because of the presence of plant growth promoting traits like nitrogen fixation, phosphorus solubilization, siderophore production and other enzymatic activities. Soil solarization integrated with other soil and seed treatments methods in order to provide protection against phytopathogens. Biopriming and soil solarization are thus two important aspects of biological control that can be utilized for strengthening seed-microbe-soil interactions. In this study, seed biopriming of tomato, chilli and capsicum seeds with suspensions of bioinoculants was carried out. These bioprimed seeds integrated with neem cake and soil solarization under field conditions reduced the seedling mortality of tomato, chilli and capsicum significantly with increase in growth parameters.

Keywords: Biopriming, biological control, endophytes, seed treatment solarization

INTRODUCTION

Healthy nursery raising is most important for solanaceous vegetable crops, as they are susceptible to a wide range of seed- and soil-borne pathogens. The pathogens associated with the damping-off disease of solanaceous crops which is devastating under nursery are soil inhabitants that can survive in soil for an indefinite period of time (Dutta, 2022). These soil-borne pathogens mainly include fungal pathogens such *as Sclerotium, Rhizoctonia, Fusarium, Colletotrichum*, and some oomycetes, such as *Pythium* and *Phytophthora*.

Economic losses of 5-80 % have been reported in established nurseries of solanaceous vegetable crops (Bhardwaj *et al.* 2023). The excess and incorrect application of chemicals in intensive agriculture has led to problems that have resulted in significant residues in agricultural products, resistance to phytopathogens, and ecological damage. Additionally, they are interfering with the amount of beneficial microorganism which is available in the soil and capable of expanding soil fertility. Nowadays, biocontrol agents assume a significant role in the field of agriculture as they are among the best alternative methods to manage the different diseases (Tariq *et al.* 2020).

Bio-priming and soil solarization are two important aspects of biological control that can be utilized to strengthen seed-microbe-soil interactions.

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Seed biopriming is an innovative and ecologically sustainable method of seed treatment that involves controlled hydration of seeds in suspensions of bio-inoculants, such as beneficial microbes, to promote growth and mitigate biotic stress (Prasad *et al*. 2020). Biopriming has emerged as the latest seed treatment method that involves a combination of physiological and biological aspects related to seed and plant growth promotion (Srivastava *et al*. 2023). The amount of water and duration of exposure of seeds to low water potential prevents actual germination, but triggers pre-germinative processes such as cell repair and protein synthesis (Devika *et al*. 2021; Sarkar *et al*. 2021).

The primary objective of promoting bio-priming technology is to reduce the use of agrochemicals and their dependence on them. Biocontrol agents such as *Trichoderma*, *Pseudomonas*, *Bacillus*, *Azospirillum*, *Azotobacter*, and *Rhizobium* can be used as potent agents for seed biopriming (Singh, 2016; Meena *et al*. 2016). Plant Growth Promoting Rhizobacteria (PGPR) are potent seed biopriming agents because of their plant growth-promoting properties, such as nitrogen fixation, phosphorus solubilization, siderophore production, and other enzymatic activities (Kumari *et al*. 2018). Several treatment methods are available for the use of bioagents, including seed or seedling treatments, foliar spraying, and soil application. Among these methods, the efficacy of seed treatment by the seed biopriming method is higher, in addition to its low requirement of microbial doses or biomass (O'Callaghan, 2016).Soil solarization is a simple, cost-effective, and inexpensive method based on the principle of entrapping solar energy. Soil solarization causes direct thermal inactivation of soil-borne pathogens, enhances soil microbial antagonism, and is a potential alternative for chemical soil disinfection (Addabbo *et al*. 2010).Soil solarization involves covering the wet soil with transparent polythene sheets in warmer areas so that it increases the soil temperature to an extent that can kill the soil-borne pathogens inhabiting the soil. The polythene sheet used for soil solarization entrapped the solar radiation inside the upper layer of the soil surface (Kanaan *et al*. 2017). The increase in soil temperature and the amount of heat accumulated determine the thermal killing effect of soil-borne pathogens. Soil solarization has proven to be an effective strategy for managing soil-borne pathogens when integrated with biological control mechanisms involving the use of fungal and bacterial antagonists by means of biopriming. The combination of soil solarization with *Streptomyces griseoviridis* was effective in managing Fusarium wilts (Minuto *et al.* 2006). Soil solarization is a sustainable technique that involves the use of warm lands to increase the soil temperature in the range of 45-55 ^{o}C at a soil depth of 5 cm, thereby inhibiting soil-borne pathogens (Thakur *et al.* 2022; Kanaan *et al.* 2015; Devendra *et al*., 2015). Soil solarization is integrated with other soil and seed treatment methods to provide protection against phytopathogens (Monterio and Santos, 2022).

MATERIAL AND METHODS

Collection and preparation of seed biopriming agents

The biocontrol agents (*Trichoderma harzianum and Trichoderma virens*) were procured from the Department of Plant Pathology, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni Solan (HP). Fungal biocontrol agents were isolated from the soil rhizosphere and were identified based upon their morphological, cultural and microscopic characteristics. Extracts of *R. elegans* and *M. azedarach* were prepared by crushing and extracting the filtrate and their evaluation was carried out using poisoned food technique (Nene and Thapliyal 2000).

Isolation of Endophytes

Fungal endophytes were isolated from stem samples of medicinal plants *Mentha* sp., *Ocimum* sp., *Vinca rosea* and *Hibiscus rosa sinensis*. Stem samples of 1 cm size were washed under tap water followed by their surface sterilization with combination of sterilants i.e. 70 %ethanol for 1 min, 5 % sodium hypochlorite for 5 min and with 70 % ethanol for 1 min. The efficacy of surface sterilization was confirmed by plating the final rinse water on PDA to check any growth. The blot dried segments were grown on PDA media in aseptic conditions and incubated

at 27°C (Khiralla *et al.* 2016).For bacterial endophyte isolation, stem segments after sterilization were grounded with 6 ml aqueous solution of 0.9 per cent NaCl using sterile mortar and pestle and thus kept as such in BOD at 28° C for 3 hrs so as to allow the complete release of endophytic microorganism. The tissue extract was plated on NA media plates after dilution of 10^{-1} and 10^{-2} and incubated at 28^0 C for 15 days. Colonies were purified again on NA media for further use (Anjum and Chandra, 2015). Fungal endophyte *Penicillium* sp. isolated from *Mentha* sp. and *Stenotrophomonas* sp. from *Ocimum* sp. were identified based on cultural and microscopic characters and subjected to molecular characterization for species conformation.

Molecular characterization of endophytes

Effective endophytes EF1 (*Penicillium* sp.) and EB5 (*Stenotrophomonas* sp.) were subjected to their molecular characterization based on 18S and 16S rRNA gene sequence analysis. After genomic DNA extraction, the isolated DNA was quantified using agarose gel electrophoresis. The amplified product obtained with universal primers (ITS1, ITS4 for fungal and 27F and 1492R for bacterial endophytes) were sent to Biokart India Pvt Ltd- Bangalore (Karnataka) India under refrigerated conditions using gel packs. Sequenced data so obtained was further analyzed using tools like BLAST (Basic Local Alignment Search Tool) of NCBI (National Centre for Biotechnology Information) for homology search and phylogenetic tree was constructed using MEGA 11 software

Seed biopriming with bioinoculants

Seed biopriming with effective bioinoculants (biocontrol agents, plant extracts and endophytes) was carried out as per the procedure followed by Singh *et al.* (2020). Seeds of tomato (var. Solan Lalima), chilli (var. DKC-8) and capsicum (var. Solan Bharpur) were obtained from Department of Seed Science and Technology, Dr. YS Parmar University of Horticulture and Forestry Nauni Solan HP. At first the concentration of suspensions of bioinoculants and duration of seed biopriming was standardized under laboratory conditions using rolled paper towel method (ISTA, 1999). In this experiment, 100 seeds were placed in each replicate for each treatment. Seeds were placed in 10 rows with 10 seeds in a row at an equal distance between two layers of moist germination paper, and covered with a layer of wax paper. These were then kept in a seed germinator and incubated at 28±2 °C for 14 days. Seed germination was recorded on the 7th day, and the final count was recorded on the 14th day. Seeds without any treatment served as controls for the comparison of treatments. The experiment was carried out in a completely randomized design, with three replicates each.

Combination of seed biopriming treatments with soil solarization under with soil amendments

Seeds of tomato, chilli and capsicum bioprimed with standardized concentration of suspensions of bioinoculants for optimized duration of time were sown separately in solarized and unsolarized nursery beds of size 1.5x1.5 m^2. Solarization was performed by covering nursery beds with thin transparent polythene sheet of 25µm (100 gauge) thickness during May–June for a period of 30 days. Half of the beds were selected for soil solarization and the remaining beds were kept unsolarized. Neem cake and vermicompost were applied at the time of preparation of nursery beds as well as during sowing of bioprimed seeds at 40 grams per m^2 concentration. The field experiment was carried out in a randomized block design with three replications for each case, and different treatment combinations were evaluated to record the incidence of damping-off disease. Data pertaining to disease incidence (%), seed germination (%), shoot length (cm) and root length (cm) were recorded for each treatment. The field experiment was carried out in randomized block design with four replicates for each treatment.

Statistical analysis

The data recorded from *in vitro* and field experiments was analyzed as the mean of three replicates and was subjected to one way ANOVA using OPSTAT statistical tool (Sheoran *et al.* 1998). The differences among treatments in different experiments were later tested for their significance and means were compared using Duncan Multiple Range Test (DMRT) of OPSTAT statistical tool.

RESULTS

Seed biopriming with bioinoculants under solarized conditions

Seeds with biocontrol agents (*Trichoderma harzianum* and *T. virens*) were primed at standardized concentration of 10^7 cfu per ml (tomato and chilli) and 10^8 cfu per ml for capsicum for 8 hrs duration and with extracts of *Roylea elegans* and *Melia azedarach* at concentration of 10 per cent for 12 hrs. However, seed biopriming of tomato, chilli and capsicum with *Penicillium dipodomyicola* was carried out at 10^8 cfu per ml and with *Stenotrophomonas* sp. at 10^7 cfu per ml for 12 hrs duration were integrated with soil amendments (neem cake and vermicompost) in 13 different combinations. The effectiveness of these combinations was then assessed in the context of controlling the damping-off disease in both solarized and unsolarized beds (Fig 1).
Treatment combination T9 in solarized plots of tomatoes had the highest efficacy in mitigating damping-off disease, resulting in a decrease in seedling mortality by 49.93 percent. The treatment combination T5 demonstrated a reduction of 43.59 percent in seedling mortality. The treatment combination T8 had the lowest level of effectiveness as compared to the control. The treatment combination T1 exhibited the subsequent highest level of effectiveness with a seed germination of 86.15 percent of tomato seeds in solarized beds, The application of treatment combination T9 resulted in a considerable enhancement of plant growth characteristics in beds subjected to solarization (Table1).

A comparative analysis of several treatments for the management of damping-off disease in chili plants revealed that solarized beds had significantly higher efficacy than unsolarized beds. Among the beds that were solarized, treatment combination T9 exhibited the highest efficacy in mitigating damping-off disease incidence, resulting in a reduction of seedling mortality by 47.21% in chilli (Table 2). This was closely followed by treatment combination T10. The results of the experiment indicate that in the context of chilli, treatment combination T3 exhibited the highest level of effectiveness in solarized beds, resulting in a seed germination of 77.47%. This was closely followed by T6 treatment, which achieved a seed germination rate of 77.33%. Seed biopriming in capsicum under solarized conditions resulted in a significant reduction of seedling mortality by 42.89 per cent as compared to the control. The treatment combination T7 had the lowest level of effectiveness, with seedling mortality of 31.50%, when compared to the control group. The treatment combination T8 demonstrated a statistically significant improvement in average shoot length (18.92 cm), followed by T3 (Table 3).

DISCUSSION

The novel endophyte, *Stenotrophomonas* sp., has been reported to be effective against soil-borne pathogens (Wolf *et al.* 2002; Mishra *et al.* 2017). However, there are no reports of endophytes such as *Penicillium dipodomyicola* and *Stenotrophomonas* sp. being used as seed biopriming agents against damping-off pathogens. The ability of *Trichoderma* spp. to suppress *Pythium aphanidermatum* in tomato seedlings has been reported previously (Khare *et al.,* 2010; Kipngeno *et al.* 2015). Tiwari *et al.* (2017) reported that application of antagonists like (*Aspergillus niger*, *Penicillium javanicum,* and *Trichoderma hamatum*) with natural plant extract *(Ocimum sanctum*, *Rauvolfia serpentina*, and *Azadirachta indica)* checked the growth of *Fusarium* by 57.14-85.71% in eggplant. The efficacy of organic amendments against soil-borne phytopathogens has been reported by research workers (Shafique *et al.* 2016; Bonanomi *et al*. 2018; Panth *et al.* 2020). The results of the present investigation corroborate the findings of various studies regarding the effectiveness of soil solarization against pathogens that cause damping-off (Raj and Bhardwaj 2000; Joshi *et al.* 2009; Uddin *et al.* 2009). Soil solarization has been reported to be effective in reducing the incidence of damping-off disease in solanaceous crops such as tomatoes, chilli, and brinjals (Pandey and Pandey 2005). Soil solarization for 30 days in tomato and chilli nurseries resulted in a lower incidence of damping-off disease (Rahman *et al.* 2003; Akhtar *et al.* 2008; Kadam *et al.* 2018).

Table1: Efficacy of seed biopriming and soil amendments integrated with soil solarization in managing damping-off disease in tomato

Treatments (Seed biopriming)	Seed germination (%)		Shoot length (cm)		Root length (cm)		Disease incidence (%)	
	Solarized beds	Un-solarized beds	Solarized beds	Un-solarized beds	Solarized beds	Un-solarized beds	Solarized beds	Un-solarized beds
T1 *Trichoderma harzianum* + Neem cake	86.15 ^{bc} (68.12)	77.01 ^{bc} (61.33)	20.49 ^{b}	10.87^{b}	10.83^{a}	8.60^{a}	18.07 ^{cf} (25.14)	30.45 ^{c} (33.48)
T2 *Trichoderma harzianum* + Vermicompost	82.78 ^{c} (65.46)	73.92 ^{cd} (59.27)	14.32 ^{ef}	9.55 ^{bcde}	8.64^{cd}	6.56^{b}	19.98 ^{de} (26.54)	33.37 ^{d} (35.27)
T3 *Trichoderma virens* + Neem cake	65.65 ^{f} (54.12)	64.34 ^{f} (53.31)	15.26 ^{cd}	8.62 ^{dc}	7.61^{c}	6.25^{b}	21.35 ^{d} (27.51)	28.19 ^{fg} (32.05)
T4 *Trichoderma virens* + Vermicompost	67.47 ^{f} (55.21)	61.33 ^{f} (51.53)	17.78 ^{cd}	8.30 ^{de}	8.28^{de}	5.24^{c}	19.60 ^{def} (26.27)	29.61^{ef} (32.95)
T5 *Roylea elegans* + Neem cake	74.17 ^{e} (59.43)	71.07 ^{de} (57.44)	15.35 ^{ef}	9.87 ^{bcd}	6.50^{f}	5.23^{c}	17.61 ^{fg} (24.79)	22.18 ^{gj} (28.08)
T6 *Roylea elegans* + Vermicompost	83.17 ^{c} (65.79)	73.90 ^{cd} (59.26)	18.53 ^{c}	10.92 ^{b}	9.13^{bc}	4.82^{c}	17.97 ^{ef} (25.07)	25.55 ^{hi} (30.31)
T7 *Melia azedarach* + Neem cake	65.98 ^{f} (54.30)	61.33 ^{f} (51.53)	16.22 ^{de}	13.00 ^{a}	6.63^{f}	5.33^{c}	20.72 ^{d} (27.06)	27.23 ^{gh} (31.44)
T8 *Melia azedarach* + Vermicompost	68.83 ^{f} (56.04)	63.91^{f} (53.06)	15.11^{ef}	10.72 ^{bc}	5.58^{g}	5.22^{c}	25.52 ^{b} (30.33)	27.17 ^{gh} (31.39)
T9 *Penicillium dipodomyicola* + Neem cake	90.85 ^{a} (72.41)	82.97 ^{a} (65.61)	24.16 ^{a}	9.64 ^{bcd}	11.48^{a}	6.45^{b}	15.63 ^{g} (23.27)	24.03 ^{ij} (29.31)
T10 *Penicillium dipodomyicola* + Vermicompost	86.74 ^{b} (68.64)	79.60 ^{b} (63.13)	13.90 ^{fg}	10.67 ^{bc}	6.53^{f}	5.53^{c}	18.00 ^{ef} (25.09)	25.57 ^{hi} (30.36)
T11 *Stenotrophomonas* sp. + Neem cake	73.45 ^{c} (58.99)	71.41 ^{de} (57.66)	14.83 ^{ef}	8.88 ^{cde}	9.45^{b}	5.56^{c}	23.50 ^{c} (28.98)	36.45 ^{c} (37.12)
T12 *Stenotrophomonas* sp. + Vermicompost	77.50 ^{d} (61.66)	69.76 ^{c} (56.62)	12.43 ^{g}	9.41 ^{bcde}	5.48^{g}	5.48^{c}	20.28 ^{d} (26.75)	38.88 ^{b} (38.56)
T13 Control	54.00 ^{g} (47.28)	44.57 ^{g} (41.86)	9.64 ^{h}	7.56 ^{e}	4.17^{h}	3.78^{d}	31.22 ^{a} (33.95)	43.16 ^{a} (41.05)
CD $_{(0.05)}$								
Treatment (T)	**1.49**		**1.22**		**0.48**		**0.93**	
Solarization (S)	**0.58**		**0.48**		**0.19**		**0.37**	
TxS	**2.10**		**1.72**		**0.68**		**1.32**	
SE (m)	**0.52**		**0.43**		**0.17**		**0.33**	
	0.20		**0.17**		**0.07**		**0.13**	
	0.74		**0.60**		**0.24**		**0.46**	
SE (d)	**0.74**		**0.60**		**0.24**		**0.46**	
	0.29		**0.24**		**0.09**		**0.37**	
	1.04		**0.86**		**0.34**		**1.32**	

Figures in parenthesis are arc sine transformed values. Figures with superscripted letters in column indicate values within the same column that are either significantly different (when letters are different) or not (when the letters are same) using DMRT

Table 2: Efficacy of seed biopriming and soil amendments integrated with soil solarization in managing damping-off disease in chilli

Treatments (Seed biopriming)	Seed germination (%)		Shoot length (cm)		Root length (cm)		Disease incidence (%)	
	Solarized beds	Un-solarized beds	Solarized beds	Un-solarized beds	Solarized beds	Un-solarized beds	Solarized beds	Un-solarized beds
T1 *Trichoderma harzianum* + Neem cake	71.00 ^{ce} (57.39)	68.02 ^{bc} (55.55)	14.75^{cd}	13.09^{bc}	9.87^{ab}	8.51^{bcd}	21.58 ^{f} (27.67)	39.75^{f} (39.07)
T2 *Trichoderma harzianum* + Vermicompost	74.27 ^{bcd} 59.49)	68.00 ^{bc} (55.53)	14.32^{d}	12.18^{cd}	8.47^{cde}	5.24^{ef}	24.53 ^{e} (29.68)	35.37 ^{hi} (36.48)
T3 *Trichoderma virens* + Neem cake	77.47 ^{a} (61.64)	65.35 ^{def} (53.92)	16.47^{b}	11.48^{cde}	7.50^{e}	7.44^{d}	27.26 ^{d} (31.46)	37.31^{gh} (37.63)
T4 *Trichoderma virens* + Vermicompost	76.13 ^{abc} (60.74)	66.48 ^{bcd} (54.61)	13.92^{d}	12.19^{cd}	8.98^{bcd}	8.77^{b}	29.37 ^{c} (32.79)	38.90 ^{fg} (38.57)
T5 *Roylea elegans* + Neem cake	73.83 ^{cd} (59.21)	68.66 ^{b} (55.94)	13.52^{d}	12.38^{cd}	10.06^{ab}	8.90^{bc}	29.17 ^{c} (32.67)	41.99 ^{e} (40.38)
T6 *Roylea elegans* + Vermicompost	77.33 ^{a} (61.55)	64.51^{def} (53.42)	13.49^{d}	11.08^{def}	8.96^{bcd}	8.60^{e}	30.61 ^{bc} (33.58)	44.27 ^{d} (41.69)
T7 *Melia azedarach* + Neem cake	65.62 ^{g} (54.08)	65.53^{cde} (54.02)	13.01^{de}	11.69^{cd}	7.87^{de}	6.25^{e}	31.59 ^{b} (34.18)	46.50 ^{c} (42.98)
T8 *Melia azedarach* + Vermicompost	68.00 ^{f} (55.53)	63.51 ^{ef} (52.82)	18.77^{a}	14.58^{ab}	9.32^{bc}	8.20^{bcd}	32.55 ^{b} (34.79)	48.77 ^{b} (44.28)
T9 *Penicillium dipodomyicola* + Neem cake	77.23 ^{a} (61.48)	74.00 ^{a} (59.32)	17.39^{ab}	15.92^{a}	10.84^{a}	10.63^{a}	18.33 ^{g} (25.34)	33.91^{ij} (35.60)
T10 *Penicillium dipodomyicola* + Vermicompost	76.67 ^{ab} (61.10)	74.67 ^{a} (59.76)	16.12^{bc}	12.48^{cd}	8.12^{de}	5.21^{ef}	19.83 ^{fg} (26.43)	30.56 ^{k} (33.55)
T11 *Stenotrophomonas* sp. + Neem cake	73.33 ^{de} (58.92)	68.17 ^{b} (55.63)	11.19^{f}	9.88^{ef}	9.56^{bc}	8.10^{bcd}	25.77 ^{de} (30.49)	36.04 ^{h} (36.88)
T12 *Stenotrophomonas* sp. + Vermicompost	76.40 ^{ab} (60.92)	64.27^{def} (53.27)	11.71^{ef}	11.45^{cde}	9.57^{bc}	7.52^{cd}	24.33 ^{e} (29.54)	32.45 ^{jk} (34.71)
T13 Control	63.77 ^{g} (52.98)	62.67 ^{f} (52.32)	10.53^{f}	9.18^{f}	7.50^{e}	4.58^{f}	34.72 ^{a} (36.09)	54.90 ^{a} (47.79)
CD $_{(0.05)}$								
Treatment (T)	**1.06**		**1.15**		**0.72**		**0.82**	
Solarization (S)	**0.42**		**0.45**		**0.28**		**0.32**	
TxS	**1.49**		**1.62**		**1.01**		**1.15**	
SE (m)	**0.37**		**0.96**		**0.26**		**0.39**	
	0.14		**0.38**		**0.10**		**0.11**	
	0.53		**1.37**		**0.37**		**0.41**	
SE (d)	**0.53**		**0.48**		**0.37**		**0.41**	
	0.21		**0.19**		**0.15**		**0.16**	
	0.74		**0.68**		**0.52**		**0.57**	

Figures in parenthesis are arc sine transformed values. Figures with superscripted letters in column indicate values within the same column that are either significantly different (when letters are different) or not (when the letters are same) using DMRT

Table 3: Efficacy of integration of seed biopriming and soil solarization in managing damping-off disease in capsicum

Treatments (Seed biopriming)	Seed germination (%)		Shoot length (cm)		Root length (cm)		Disease incidence (%)	
	Solarized beds	Un-solarized beds	Solarized beds	Un-solarized beds	Solarized beds	Un-solarized beds	Solarized beds	Un-solarized beds
T1 *Trichoderma harzianum* + Neem cake	75.78 ^{bc} (60.49)	71.02 ^{a} (57.41)	15.45 ^{b}	13.78 ^{ab}	8.81 ^{c}	8.35 ^{ab}	25.17 ^{cd} (30.09)	30.83 ^{fg} (33.71)
T2 *Trichoderma harzianum* + Vermicompost	74.85 ^{cd} (59.22)	71.10 ^{a} (59.61)	14.67 ^{bc}	10.81 ^{de}	8.55 ^{cd}	5.94 ^{c}	27.98 ^{bc} (31.92)	49.58 ^{a} (44.71)
T3 *Trichoderma virens* + Neem cake	73.92 ^{d} (59.27)	71.22 ^{a} (57.53)	15.72 ^{b}	11.78 ^{cd}	7.28 ^{d}	7.48 ^{b}	26.41 ^{bc} (30.91)	46.05 ^{ab} (42.72)
T4 *Trichoderma virens* + Vermicompost	70.72 ^{e} (57.23)	50.36 ^{h} (45.19)	13.69 ^{c}	10.72 ^{de}	9.41 ^{bc}	8.58 ^{ab}	28.70 ^{abc} (32.38)	34.42 ^{ef} (35.91)
T5 *Roylea elegans* + Neem cake	67.50 ^{f} (55.22)	64.07 ^{c} (53.16)	13.38 ^{cd}	11.79 ^{cd}	10.98 ^{a}	8.11 ^{ab}	26.67 ^{bc} (31.07)	39.87 ^{cd} (39.14)
T6 *Roylea elegans* + Vermicompost	75.10 ^{c} (60.04)	61.17 ^{d} (51. 43)	12.12 ^{df}	10.82 ^{de}	8.24 ^{cd}	7.94 ^{b}	30.36 ^{abc} (33.42)	31.44 ^{fg} (34.09)
T7 *Melia azedarach* + Neem cake	58.33 ^{g} (49.78)	58.15 ^{e} (49.67)	13.37 ^{cde}	11.68 ^{cd}	7.31 ^{d}	5.65 ^{c}	31.50 ^{ab} (34.13)	41.59 ^{bcd} (40.14)
T8 *Melia azedarach* + Vermicompost	57.11 ^{h} (49.07)	55.25 ^{g} (47.99)	18.92 ^{a}	13.56 ^{b}	10.31 ^{ab}	8.62 ^{ab}	31.00 ^{ab} (33.82)	37.34 ^{de} (37.65)
T9 *Penicillium dipodomyicola* + Neem cake	79.17 ^{a} (62.82)	57.04 ^{f} (49.03)	14.89 ^{bc}	14.72 ^{a}	8.73 ^{c}	7.43 ^{b}	19.06 ^{e} (25.88)	26.50 ^{g} (30.97)
T10 *Penicillium dipodomyicola* + Vermicompost	76.43 ^{b} (60.94)	69.44 ^{b} (56.42)	13.48 ^{c}	12.70 ^{bc}	8.99 ^{c}	5.22 ^{c}	20.43 ^{de} (26.87)	26.02 ^{g} (30.66)
T11 *Stenotrophomonas* sp. + Neem cake	71.55 ^{e} (57.74)	61.34 ^{d} (51.54)	11.18 ^{f}	10.89 ^{de}	9.44 ^{bc}	9.91 ^{a}	26.56 ^{bc} (30.92)	32.86 ^{ef} (34.96)
T12 *Stenotrophomonas* sp. + Vermicompost	76.87 ^{b} (61.25)	70.21 ^{ab} (56.90)	11.82 ^{f}	11.14 ^{cd}	8.73 ^{c}	8.41 ^{ab}	27.63 ^{bc} (31.70)	32.50 ^{ef} (34.74)
T13 Control	53.67 ^{I} (47.09)	50.78 ^{h} (45.43)	9.51 ^{g}	8.56 ^{c}	7.34 ^{d}	5.22 ^{c}	33.38 ^{a} (35.28)	42.55 ^{bc} (40.69)
CD $_{(0.05)}$								
Treatment (T)	**0.77**		**0.88**		**0.75**		**2.01**	
Solarization (S)	**0.30**		**0.34**		**0.29**		**0.79**	
TxS	**1.09**		**1.24**		**1.07**		**2.85**	
SE (m)	**0.28**		**0.31**		**0.27**		**0.71**	
	0.11		**0.12**		**0.10**		**0.28**	
	0.40		**0.44**		**0.37**		**0.99**	
SE (d)	**0.40**		**0.44**		**0.37**		**0.99**	
	0.16		**0.17**		**0.15**		**0.39**	
	0.57		**0.62**		**0.53**		**1.41**	

Figures in parenthesis are arc sine transformed values. Figures with superscripted letters in column indicate values within the same column that are either significantly different (when letters are different) or not (when the letters are same) using DMRT

Fig.1 : Efficacy of soil solarization integrated with seed biopriming against damping-off disease under field conditions

Rahman *et al.* (2003) reported that soil solarization resulted in lowest incidence of damping-off in tomato (3.9 %) and chilli (1.3 %) in comparison to control. The increase in soil temperature to approximately 7.7°C has been considered a major driving force for different types of biological changes in the soil, as reported in the case of soil solarization. Pandey *et al.* (2014) reported that soil solarization treatment was most effective in managing chickpea wilt in comparison to Farm Yard Manure with increase in shoot and root growth of chickpea plants. Solarization treatments effectively increased the soil temperature at a depth of 5 cm. Solarization integrated with organic amendments, such as vermicompost and FYM, changed the microbiota of the soil and significantly reduced the fungal and bacterial populations (Mullalmaran*et al.* 2019). Neem cake has been reported to have nematicidal and bacterial activities (Goswami and Mittal, 2004). Neem products have suppressive effect against the phytopathogens due to the production of fungistatic substances as azadirachtin and thus reported to suppress the growth of *Fusarium oxysporum* f. sp. *lycopersici* (Kimaru*et al.* 2004). The incidence of damping-off in white polythene sheets was significantly reduced in tomatoes, cauliflowers, and onion nurseries (Minuto *et al.* 2000). In view of increasing concerns regarding the conservation of natural resources and residual toxicity of hazardous chemical pesticides, soil solarization has proven to be an absolute technology in integration with seed biopriming to combat soil-borne plant pathogens in a sustainable manner with no harmful effects on the soil and environment (Khulbe, 2019).

Soil solarization has been reported to increase the sensitivity of soil pathogens to the toxic effects of volatiles, such as ammonia, methanehiol, dimethyl sulfide, allyl isothiocyanates, and aldehydes. Soil solarization for 40 days has been reported to reduce the viability of *Fusarium oxysporum* and *Rhizoctonia solani*, which causes a complete loss of pathogen viability (Reddy *et al.* 2006; Bhardwaj *et al.* 2023). Reduction in soil-borne pathogens offers a satisfactory tool for Integrated Disease Management. Seed biopriming with endophytic microorganisms could be utilized as an eco-friendly approach in order to mitigate the resistance development problem as the endophytes inherit properties like phytohormone production, induction of resistance and tolerance to biotic and abiotic stresses (Kumar *et al*. 2020). Biopriming plays an important role in improving germination and viability of seed, growth and yield of plants (Prasad *et al*. 2016; Bhatt *et al*. 2015). Root endophyte *Piriformospora indica* is effective in increasing grain productivity of barley due to its resistance attributes (Waller *et al*. 2005). Seed biopriming with endophytic species of *Acremonium, Serratia*, *Pseudomonas*, *Fusarium*, *Neotyphodium* have been reported to enhance seed germination, nutrient supply and plant growth (Furnkranz *et al.* 2012; Aime *et al.* 2015; Van Hecke *et al.* 2005). Seed biopriming with endophytic fungus *Epichloe* on the growth of Festuca sinensis under green house has significant improvement for seedling germination, seed vigour index, number of leaves as well as dry weight of seedlings (Peng *et al.* 2013). Seed biopriming with endophytic bacteria *Bacillus subtilis* strain 11BM was found effective in increasing root and shoot weight of wheat seeds due to accumulation of indole acetic acid, indole butyric acid in seedlings of root as well as shoot. Combined treatment with biocontrol agent and endophytic fungi *Aspergillus tereus* strain 2aWF,

Penicillium oxalicum strain 5aWF and *Sarocladium kiliense* strain 10aWF in *Withania somnifera* resulted into an increase in shoot weight, root weight and plant height (Kushwaha *et al*. 2019). The interactive effects of different priming techniques with endophytic microbe based biopriming could provide better outcomes for the management of crop productivity. Biopriming with endophytic *Bacillus subtilis* MA17 offered highest plant growth promotion and salinity tolerance and bio protection against fusarium head blight (Brahim *et al*. 2022).

CONCLUSION

Microbial inoculants have significant role in promoting ecological and agricultural sustainability. The comprehension of plant-microbe interactions contributes to reduction in reliance on chemical inputs for disease management. Soil solarization is an environmental-friendly approach of disease management. Seed biopriming is an innovative approach to disease management that possesses the capability to induce physiological and biochemical alterations in seeds. Additionally, this approach enhances the protection of seeds from infections present in the soil and those that are seed-borne. The presence of a microbial coating on seeds promotes the interaction between beneficial microorganisms, soil microbiomes, and microflora. Therefore, it can be inferred from the present study that the utilization of seed biopriming in integration with soil solarization under neem cake amended soil can be used as highly effective strategy in promoting sustainable agriculture with reduction in incidence of damping-off disease. However, despite the increasing need for the utilization of environmental friendly approaches of disease management, there exist specific hurdles in implementing seed biopriming as a commercially viable method for disease management. The challenges pertaining to the commercial formulations, dosage, and viability are the concerns which are associated with these that needs to be acknowledged and tackled.

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