

Original Research Article

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Integration of Soil Solarization, Arbuscular Mycorrhizal Fungi, *Trichoderma viride*, *Azotobacter chroococcum* and Soil Amendments for the Management of Carnation (*Dianthus caryophyllus* L.) Wilt (*Fusarium oxysporum* f.sp. *dianthi* (Prill and Del.) Snyd. and Hans.)

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ABSTRACT

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Studies were conducted on Carnation (*Dianthus caryophyllus* L.) to find out the effect of integrated inoculation of potent native isolates of Arbuscular mycorrhizal fungi (AM fungi) and *Azotobacter chroococcum* with other approaches of management like cultural and biological methods in solarized soil on the incidence of wilt caused by *Fusarium oxysporum* f.sp. *dianthi* (Prill. and Del.). Initially, organic amendments, botanicals and bio-control agents were evaluated against wilt to find out the best treatments. Among amendments, neem cake was found most effective with 75.0 per cent reduction in the wilt incidence. Different fungicides, botanicals, bio-pesticides and bio-control agents were also evaluated against wilt by dip treatment of unrooted carnation cuttings. Bavistin among fungicides, Neemazal among botanicals and *Trichoderma viride* among bio-control agents were found effective with 100.0, 71.1 and 93.6 per cent reduction in the wilt incidence. Based on the best individual treatments, fourteen treatment combinations were evaluated in solarized and unsolarized plots for their efficacy against the disease. Among different treatments, root dip of cuttings in Bavistin (0.1%), soil amendment with Neemcake (1kg/m²), root inoculation with culture of AM fungi and *A. chroococcum* (5g culture/plant) and soil application of *T. viride* formulation (10g/m²) in solarized soil was found most effective with 97.1 per cent reduction in the wilt incidence. This treatment combination also resulted in maximum increase of 50.97, 100.4, 39.2, 57.3, per cent in plant height, number of flowers per plant, flower size and length of flowering stem, respectively in comparison to control.

Introduction

Vascular wilt caused by *Fusarium oxysporum* f.sp. *dianthi* is most prevalent disease in carnation and upto 79 per cent incidence has been recorded in different parts of the Himachal Pradesh (Chandel and Katoch,

2001). Soil-borne pathogens are difficult to control due to repeated cultivation of the crop in the same piece of the land. Use of chemicals in the management of soil-borne disease results in high cost of production and also has drastic adverse effect on soil microflora (Aktar *et al.*, 2009). Thus, there is

an urgent need for development of integrated disease management strategy by evaluation of other physical, biological and cultural methods effective against the wilt pathogen. Soil solarization (SS) is one of the important and cost-effective methods for the management of soil-borne pathogens in different crops in different regions (Katan 1981). Bio-control agents and soil amendments have also been reported effective against the soil-borne diseases (Lodha and Israel 2005, Karimi *et al.*, 2007). Soil is rich in many beneficial microorganisms like VA mycorrhizal fungi and *A. chroococcum* which are beneficial to the plants in enhancing plant growth and productivity, and these organisms also help in reducing incidence of different soil-borne pathogens (Smith, 2002, Dehne, 1982 and Brown, 1974). Soil solarization has been found more effective against soil-borne pathogens when integrated with biological control agents, soil amendments and chemical treatment (Gamliel and Stapleton 1993, Raj and Sharma 2009). Hence, the present investigation was undertaken to evaluate the integrated efficacy of SS, botanicals, bio-control agents, soil amendments and chemicals for management of the disease

Materials and Methods

Soil amendments

Soil amendments were evaluated to find their effect on the incidence of *Fusarium* wilt of carnation and to know their effect on important plant growth characteristics and quality parameters of the flowers. In this trial, seven soil amendments viz., neem (*Azadirachta indica* L.) cake, vermin-compost, darek (*Melia azedarach* L.) seed meal, karu (*Roylea elegans* Wall.) leaves, cauliflower (*Brassica oleracea* L. var. *botrytis* L.) leaves and banna (*Vitex negundo*) leaves were used at the rate of 100 g/pot which contained 5 kg of soil. In addition, neem

granules (Azadirachtin 0.15 % (E.I.D. Parry (India) Ltd.) were also used and were applied at the rate of 10 g/pot. These amendments were mixed thoroughly in the upper 15cm soil layer. Soil was then irrigated to saturation level and left for the decomposition for two weeks before planting the carnation cuttings.

Root dip treatments

Unrooted carnation cuttings of variety 'Sunrise' were dipped in different treatments of fungicides, botanicals and bio-pesticides for 30 minutes followed by a quick dip with NAA at 500 ppm before planting them into rooting media containing sand and soil in the ratio of 1: 1. Fungicides viz., carbendazim (Bavistin 50% WP) (0.1%), hexaconazole (Contaf 5% EC) (0.05%), difenoconazole (Score 25% EC) (0.025%), mancozeb (Dithane M-45 75% WP) (0.25%), iprodione 25% + carbendazim 25% WP (Quintal) (0.2%), carbendazim 12% + mancozeb 63% WP (Saaf) (0.2%), captaf (captan 50% WP) (0.2%), pyraclostrobin 5% + metiram 55% WG (Cabrio Top) (0.2%) and myclobutanil (Systhane 10% WP) (0.05%) were taken. In botanicals and bio-pesticides plants like darek (*Melia azedarach* L.) (1%), karu (*Roylea elegans* Wall.) (1%), dudhli (*Cryptolepsis buchanani* Roem. & Schult.) (1%), tulsi (*Ocimum sanctum* L.) (1%) shambri (*Artemisia roxburghiana*) (1%), safeda (*Eucalyptus globulus*) (1%), gharit kumari (*Aloe vera*) (1%), commercial formulation of neem (Neemazal 1.0% EC) (1%) and also vermiwash (1%) were taken. In fungal antagonists like *Trichoderma viride* (1%) and *T. harzianum* (1%) were taken where one per cent formulation was made by taking one gm of commercially available formulation made in talc powder and then dissolving it in 100 ml water. In bacterial antagonists, *Bacillus subtilis* (1%), *Brevibacillus brevis* (1%), *Azotobacter chroococcum* (1%) and *Pseudomonas fluorescens* (1%) were taken

where one per cent formulation was made by dissolving 1ml of Nutrient Agar broth culture of bacteria in 100 ml water. Cuttings were inserted in the rooting media upto two nodes and then kept in the mist chamber. Data on disease incidence, root length and plant height were recorded after 30 days.

Isolation, identification and mass multiplication of native potent isolates of AM Fungi and *A. chroococcum*

Soil samples were collected from different carnation growing areas of the State to isolate potent isolates of AM fungi. Seven potent isolates, viz *Glomus mosseae*, *G. fasciculatum*, *G. macrocarpum*, *G. constrictum*, *Acaulospora bireticulata*, *Gigaspora* sp., *Entrophospora* sp. were selected on the basis of occurrence and frequency of distribution in the carnation growing areas. The consortium of these seven potent isolated isolates of AM fungi was made and named as AMUHF. The AMF spores were isolated by wet sieving and decanting method of Gerdemann and Nicolsan (1963) and identified to the genus level under tri-nocular biological microscope (Leica DMLB) attached with a digital camera. Spores were identified by different synoptic keys (Morton 1988). These isolates were multiplied on green gram (*Vigna radiata* L. Wilczek) in sterilized soil in earthen pots for 3 months. These plants were uprooted after 3 months and their roots were chopped into pieces to develop mass culture of consortium of AM fungi for inoculation into soil. The inoculum of different isolates used in the field experiments contained spores of the isolate, pieces of infected chopped roots and mycelium in the pot culture soil. Isolate of *A. chroococcum* was selected from the rhizosphere soil of carnation by serial dilution technique and it was named as AZUHF. 10g soil from the rhizospheric soil of carnation was drawn and serially diluted aseptically to

10^{-3} , 10^{-4} , 10^{-5} and 10^{-6} dilutions and out of this 1ml of suspension was spreaded on Jenson's medium (Subba Rao, 1986). Culture carrier of each isolate was prepared in 10 % jaggery slurry added with gum to stick. This slurry of the culture was prepared to apply the culture to the roots.

Soil solarization

Soil solarization was done for 40 days during 1st May to 9th June 2011 with thin transparent polyethylene sheet (25 μ m thick). Beds (1 x 1m) were irrigated to saturation level and then covered with thin transparent polyethylene sheet. The sheets were removed after 40 days of solarization. In the second set, beds were not covered with any sheet and served as control for comparison. During the period of solarization, soil temperature was recorded every day for 40 days at 2 pm in both solarized as well as unsolarized beds with dial type digital thermometer at 5 and 15 cm soil depths.

Integrated disease management

Treatments that proved effective under *in vitro* and polyhouse experiments were then integrated with soil solarization to know and compare their individual and combined effect on the incidence of carnation wilt. The experiment was laid out in the polyhouse during the year 2011, which comprised of effective treatments of soil amendments, root dip/treatment of cuttings with fungicides /botanicals/bio-pesticides/bio-control agents and effective combination of AM fungi, *Azotobacter chroococcum* and *T. viride* in different combinations in solarized and unsolarized soil. Talc based formulation of *T. viride* was applied before planting @ 1% i.e. by mixing 10 g of the talc powder formulation (6×10^6 cfu/g) in 1kg well rotten farm yard manure per bed. Neem cake was applied at the rate of one kg/m² both in solarized and

unsolarized beds (1m x 1m) and were mixed thoroughly in the upper 15 cm soil layer irrigated to saturation level and left for the decomposition for two weeks. The roots of the carnation cuttings were dipped for 15 minutes in culture slurry of the *A. chroococcum* so that bacteria could adhere on the root surface. Among fungicides, bavistin (0.1%) was used as root dip treatment of the cuttings for 30 minutes and among botanicals/bio-pesticides, Neemazal was used at 20 per cent concentration as root dip treatment of cuttings for 30 minutes before planting. The carnation cuttings were planted in solarized and unsolarized beds in planting holes which were added with 5g inoculum of AMUHF before planting. Recommended dosages of chemicals fertilizers used in the polyhouse experiments were urea (46% N), single super phosphate (16% P₂O₅, 19% Ca, 12% Sulphur) and muriate of potash (60% K₂O).

IDM treatments

Fourteen treatments, viz T₁, root dip of cuttings in Bavistin @ 0.1 %; T₂, soil application of *T. viride* @ 10g/1 kg of FYM; T₃, root dip of cuttings in Neemajal @ 20 %; T₄, soil amendment with Neem cake @ 1 kg/m²; T₅, (T₄ + T₂); T₆, (T₄ + T₁); T₇, (T₄ + T₃); T₈, root inoculation of cuttings with AMUHF @ 5g/plant + AZUHF @ 5g/plant + Soil application of *T. viride* @10g/1kg of FYM/m²; T₉, (T₄ + T₈); T₁₀, (T₁ + T₈); T₁₁, (T₃ + T₈); T₁₂, (T₁ + T₄ + T₈); T₁₃, (T₃ + T₄ + T₈) and T₁₄ Control (Unamended and unsolarized) were applied in the field in the poly-house each comprising of three replications in Randomized Block Design. Carnation cuttings of variety 'Master' were planted at a distance of 20 x 20 cm in 1m x 1m bed with 25 cuttings per bed. Per cent disease incidence was calculated during the growing period in each bed. Data pertaining to plant growth and quality parameters viz., plant

height (cm), number of days taken for first flowering (days), number of flowers per plant, length of flowering stem (cm) and flower size (cm) were recorded by selecting 5 plants per replication in each treatment.

Statistical analysis

The data recorded from pots and mist chamber experiments were analyzed as per the procedure of Completely Randomized Design (CRD) and data of field experiments were statistically analyzed using Randomized Block Design (RBD) as described by Gomez and Gomez (1984). Least significance difference at 5% level was used for testing significant differences. The data on per cent disease incidence were arc sine transformed (in parentheses) then subjected to statistical analysis.

Results and Discussion

Effect of soil amendments

Soil amendments were found effective in reducing the incidence of the wilt. However, neem cake was found most effective among all the treatments which resulted in 16.67 per cent reduction in the incidence of wilt in comparison to 66.67 per cent in control. This treatment also resulted in maximum increase (44.7 %) in plant height and took 8.9 per cent less days to 1st flowering. Soil amendments have been reported to enhance the activity of the soil microflora which are potentially competitive or antagonistic against several soil-borne pathogens by different modes of actions including production of various biochemical substances during decomposition (Hortink and Fahy 1986). Negi (2009) reported that soil amendment with neem cake was found effective with 35.4 per cent reduction in incidence of wilt of carnation. Soil amendment with neem cake has also been reported most effective with 71.0 per

cent reduction in incidence of *Fusarium* wilt (*F. oxysporum* f.sp. *dianthi*) of carnation (Chandel, 2011). The mechanism of disease control for high nitrogen containing amendments like oil cakes is the generation of ammonia and nitrous acid following degradation of the amendments by microorganisms which is lethal to pathogens (Lazarovits *et al.*, 2001). Application of nitrogen rich soil amendments (oil cakes) reduced soil-borne diseases by releasing allelochemicals (Bailey and Lazarovits, 2003) (Table 1).

Effect of root dip treatments

Among fungicides, dip of carnation cuttings in Bavistin and Quintal were found most effective with complete reduction of incidence of wilt. However, treatment with Bavistin also resulted in maximum increase of 66.0 and 440.5 per cent in average plant height and root length followed by Quintal with 54.1 and 372.7 per cent, respectively in comparison to control (Table 2). Kishore and Kulkarni (2008) also reported effectiveness of carbendazim against *Fusarium* wilt of carnation. Drenching of rooting media of carnation with carbendazim @ 0.2% has also been reported effective in reducing the incidence of *Fusarium* wilt of carnation (Sharma 2000).

Among bio-control agents, *T. viride* has been found most effective with 2.3 per cent incidence of wilt followed by *A. chroococum* with 6.3 per cent disease incidence. Treatment of the cuttings with *T. viride* resulted in maximum increase of 73.1 and 586.4 per cent in average plant height and root length followed by *A. chroococum* with maximum average increase of 63.2 and 575.7 per cent, respectively in comparison to control (Table 3). Chandel (2011) reported that root dip of carnation cuttings in *T. viride* is effective with 68.55 per cent reduction in

the incidence of wilt in comparison to control. *T. viride* and *T. harzianum* applied during rooting of carnation cuttings strongly promoted growth of plants and gave good control of *F. oxysporum* f.sp. *dianthi* (Manka *et al.*, 1997; Weber *et al.*, 1998). Martinez and Pinzon (1999) also reported that application of *Trichoderma* spp. to unrooted carnation cuttings at the time of application of rooting hormone and one more application to the soil immediately before planting resulted in reduction in incidence of *Fusarium* wilt.

Among different treatments of the botanicals and bio-pesticides, dip of cuttings in Neemajal was found most effective with 11.9 per cent incidence of the wilt. Further, treatment of unrooted carnation cuttings with Neemajal resulted in maximum increase of 597.6 and 51.9 per cent in average root length and plant height in comparison to control. Chandel and Tomar (2008) reported that dip of carnation cuttings in neem formulation (Achook) is most effective with 89.6 per cent reduction in the incidence of wilt (*F. oxysporum* f.sp. *dianthi*) in carnation followed by Neemajal in comparison to control. The mechanism behind the disease control may be the Azadirachtin from neem which act as a chitin inhibitor and cause lysis of cell walls of resting pathogenic spores present in sick soil and stimulation of fungal antagonist in soil may have an indirect effect (Bhattacharya and Pramanik, 1998) (Table 4).

Effect of soil solarization

Soil solarization with transparent polyethylene sheet resulted in average increase of 8.3 °C in the soil temperature at 5 cm soil depth with average maximum soil temperature of 41.0°C in the solarized soil in comparison to unsolarized beds (Table 5). However, increase in average maximum soil temperature at 15cm soil depth was 6.0°C. In general, transparent polyethenes mulch (25µm

thick) has been reported to be effective in increasing the average maximum soil temperature (Katan, 1981). Melero-Vara *et al.*, (2005) also reported increase of 5-7 °C in average maximum temperature in the poly-house in an experiment on use of soil solarization for the management of *Fusarium* wilt of carnation.

Effect of IDM on disease incidence and growth characteristics

Integration of different effective treatments had enhanced efficacy than the individual treatments in the management of the wilt and in the improvement of plant growth and flower quality characteristics in carnation. All the treatment combinations were found effective and these treatments were more effective under solarized conditions (Table 6 and 7). These treatments reduced the wilt incidence ranging from 54.2 to 97.2 per cent under solarized plots in comparison to 51.5 to 80.0 per cent under unsolarized plots. Treatment combination T₁₂ (root dip of carnation cuttings in Bavistin @ 0.1% + Neemcake @ 1kg/m² as soil amendment + AMUHF @ 5g/plant as soil application + AZUHF @ 5g/plant as root inoculation of cuttings and *T. viride* @ 10g/1kg of FYM/m² as soil application) in solarized plots was found most effective with wilt incidence of 1.3 per cent in comparison to 46.6 per cent in unsolarized control (Table 6). Different components of the Treatment T₁₂ have a distinctive effect in enhancing the efficacy of the treatment. All the treatment combinations were found statistically superior in solarized soil with 2.7 to 17.2 per cent more control in the incidence of the wilt. Soil solarization has been reported effective for the management of wilt of carnation (Melero-Vara *et al.*, 2005). Reduction in disease incidence due to the application of organic amendments with solarization has been reported in *Fusarium* and *Phytophthora capsici* infestation in

pepper (Martínez *et al.*, 2011; Núñez-Zofio *et al.*, 2011) and was at least partially attributed to the production of NH₃ and an increase in soil microbial activity, which can help control soil-borne pathogens through competition, antibiosis, parasitism/predation, etc. (Núñez-Zofio *et al.*, 2011). Soil solarization in combination with soil amendments, crucifer residues and microbial pesticides like *Trichoderma* spp., *Gliocladium* sp., *Pseudomonas* sp. has also been reported to be effective in strawberry, gladiolus, vegetables and other crops against different soil-borne diseases (Porras *et al.*, 2009, Raj and Upmanyu, 2013).

In Treatment combination T₁₂, root inoculation with culture of AM fungi and *A. chroococcum* have a significant effect in the management of the wilt. If we compare treatments T₆ and T₁₂, it is evident that addition of T₈ with T₆ resulted in 11.4 per cent more reduction in the incidence of the wilt. There are number of reports in the literature which explain the role of AM fungi, *A. chroococcum* and *Trichoderma* spp. in the management of different soil-borne diseases. Inoculation of carnation cuttings with *Glomus intraradices* has been reported to reduce *Fusarium* wilt (*F. oxysporum* f.sp. *dianthi*) and the reduction in disease incidence was associated with reduction of number of propagules of the wilt pathogen. Reduction in the wilt incidence has been attributed either to the induction of disease resistance mechanism by the mycorrhizal fungus, or by direct/indirect interaction between VAM fungus and *F. oxysporum* f.sp. *dianthi* inoculum in the soil (St-Arnaud *et al.*, 1997). Application of VA-mycorrhizal fungi *Gigaspora margarita* in pea against *Fusarium* wilt (*Fusarium oxysporum* f.sp. *lisi*) resulted in minimum incidence (10.8%) of wilt in comparison to 45.8 per cent in control (Verma and Dohroo 2005). VA-mycorrhizal fungi exert number of factors, like lignifications of

mycorrhizal roots, increased respiration, increased production of arginine and isoflavonoids, better Phosphorus nutrition, changes in the antioxidant enzymes, phenolic compounds and pathogen related-proteins which are reported to contribute in imparting resistance against soil-borne pathogens (Dehne *et al.*, 1978; Dehne, 1982; St-Arnaud *et al.*, 1994; Morandi, 1996; Khallal, 2007).

Efficacy of *Trichoderma* spp. in different treatment combinations has been reported against different soil-borne diseases. Dipping of corms of gladiolus in carbendazim (0.05 %) for 30 minutes along with soil application of neem cake (100 g per row) and *T. viride* (2.5 % w/w) has been resulted in 74.51 per cent reduction in disease incidence of *Fusarium* yellows (*Fusarium oxysporum* f.sp. *gladioli*) in gladiolus in comparison to control (Sharma *et al.*, 2005). Inoculation of four AMF (*Glomus intraradices*, *Glomus mosseae*, *Glomus claroideum* and *Glomus constrictum*) and *Trichoderma* sp. in the seedlings nurseries has been reported to reduce the incidence of *Fusarium* wilt (*Fusarium oxysporum* f.sp. *melonis*) in melon seedlings (Martinez-Medina *et al.*, 2009). Integration of SS, *Glomus fasciculatum* isolate of Vamycorrhiza and native isolate of *A. chroococcum* was found most effective with no incidence of white root rot of apple caused by *Dematophora necatrix* in comparison to 33.6-35.4 per cent in control (Raj and Sharma, 2009). Tomato seedlings inoculated with *T. harzianum* and arbuscular mycorrhizal fungi (AMF) has been reported to have reduced disease severity of wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* (Mwangi *et al.*, 2011). Integration of SS along with application of neem cake (30q/ha) and *Azotobacter* (40kg/ha) has been resulted in 44.3 per cent reduction in the disease incidence of wilt (*Fusarium oxysporum* f. sp. *cumini*) of cumin in comparison to control (Bijarniya and Lal, 2009). *A. chroococcum*

has been reported to have antagonistic effect against *Fusarium oxysporum* by degradation and digestion of cell wall components, empty cell (halo) formation, shrinking and lysis of fungal mycelia along with significant degeneration of conidia (Maheshwari *et al.*, 2012). Similarly, many researchers have reported inhibitory effects of *A. chroococcum* on different soil-borne diseases (Ebtehag *et al.*, 2009; Umesh and Mane, 2010).

Effect of IDM on quality parameters

Different treatment combinations also resulted in improvement of important plant growth and quality characteristics of carnation both in solarized and unsolarized plots. Treatment combination T₁₂ in solarized plots was found most effective with an increase of 50.97, 100.4, 39.2 and 57.3 per cent in plant height, number of flowers per plant, flower size and length of flowering stem, respectively and also recorded 15.22 days to 1st flowering, respectively in comparison to unsolarized and unamended control (Table 2 and 3). Different components of Treatment combination T₁₂ have been reported to have positive effect on different growth and quality characteristics of different plants raised in soil infected by different soil-borne pathogens. SS has been reported to support higher growth and yield in different crops including nursery of fruits and vegetables (Patel, 2001; Raj, 2004). The mechanism for explaining increased growth responses and yield in plants has been attributed to chemical factors (like release of nutrients and other growth factors, nullification of toxins) and biological factors (elimination of minor or unknown pathogens) and stimulation of beneficial micro-organisms (Stevens *et al.*, 2003). Gawande *et al.*, (2001) reported that SS and *Trichoderma* sp. resulted in recording the least number of days required for flower bud initiation and first flower bud opening per plant in chrysanthemum. Inoculation of

mango seedlings with *Glomus fasciculatum* and *A. chroococcum* in solarized soil has been reported to increase seedlings height, diameter, leaf area, total root length, leaf N, P, K and Zn content in comparison to control (Sharma *et al.*, 2011). The direct mechanisms of increase in root development and plant growth by *Azotobacter* has been attributed to the secretion of vitamins and amino acids; production of siderophores and auxins (Akbari *et al.*, 2007). Similarly, conjoint inoculation of plants with *Azotobacter* and Va-mycorrhizae has been reported to increase

in the rhizosphere populations of bacteria and actinomycetes and resulted in synergistic growth enhancement of the host plant (Bagyaraj and Menge, 1978). Thus, root dip of cuttings in Bavistin (0.1%), soil amendment with Neemcake (1kg/m²), root inoculation with culture of AM fungi and *A. chroococcum* (5g culture/ plant) and soil application of *T. viride* formulation (10g/m²) in solarized soil is effective with 97.1 per cent reduction in the wilt incidence.

Table.1 Effect of different organic amendments on the incidence of *Fusarium* wilt and important plant growth characters

Treatments	(Rate of application in g/ 5kg of pot soil)	Disease incidence (%)	Plant height (cm)	Number of days taken for 1 st flowering
Neemcake	100g	16.67 (19.99)	75.21	128.67
Cauliflower leaves	100g	33.33 (34.99)	70.97	131.00
Neem granules	5g	25.00 (24.99)	73.15	129.33
<i>Melia azedarach</i> (S)	100g	33.33 (34.99)	68.44	133.67
<i>Roylea elegans</i> (L)	100g	41.67 (39.98)	66.20	133.00
<i>Vitex negundo</i> (L)	100g	58.33 (49.98)	62.34	134.67
Vermicompost	100g	50.00 (44.98)	64.57	135.33
Control	–	66.67 (59.97)	51.95	141.33
CD _{0.05}		NS	5.48	6.7

S, Seed meal; L, Leaves

* Figures in parentheses are arc sine transformed values

Table.2 Effect of dip treatment of unrooted carnation cuttings in fungicides on the incidence of *Fusarium* wilt and important plant growth parameters

Fungicides	Conc. (%)	Diseases incidence (%)		Mean	Plant height (cm)		Mean	Root length (cm)		Mean
		2010	2011		2010	2011		2010	2011	
Bavistin	0.1	0 (0)	0 (0)	0 (0)	16.63	18.11	17.37	6.43	6.64	6.54
Dithane M-45	0.25	17.45 (4.17)	15.87 (3.97)	16.66 (4.07)	13.87	14.27	14.07	3.41	3.56	3.49
Score	0.025	11.11 (3.32)	11.11 (3.32)	11.11 (3.32)	13.06	13.3	13.18	3.16	3.21	3.19
Contaf	0.05	15.87 (3.97)	20.63 (4.54)	18.25 (4.25)	12.98	13.02	13.00	2.50	2.79	2.64
Quintal	0.2	0 (0)	0 (0)	0 (0)	15.97	16.27	16.12	5.53	5.92	5.72
Saaf	0.2	6.35 (2.48)	1.59 (0.73)	3.97 (1.61)	15.72	15.23	15.47	5.43	5.59	5.51
Captaf	0.2	20.63 (4.54)	19.04 (4.34)	19.83 (4.44)	12.22	12.78	12.50	2.34	2.16	2.25
Systhane	0.05	25.39 (5.03)	26.98 (5.19)	26.19 (5.11)	11.61	11.51	11.56	1.76	2.28	2.02
Cabrio Top	0.2	9.52 (3.09)	11.11 (3.32)	10.31 (3.2)	13.01	13.93	13.47	3.27	4.19	3.73
Control	–	30.15 (5.47)	25.39 (5.01)	27.77 (5.24)	10.28	10.64	10.46	1.44	0.98	1.21
Mean		13.65 (3.21)	13.17 (3.04)		13.54	13.91		3.53	3.73	
CD _{0.05}		0.53			0.77			0.67		

* Figures in parentheses are arc sine transformed values

Table.3 Effect of dip treatment of unrooted carnation cuttings in different bio-control agents on the incidence of *Fusarium* wilt and important plant growth parameters

Biocontrol agents	Con c. (%)	Diseases incidence (%)		Mean	Plant height (cm)		Mean	Root length (cm)		Mean
		2010	2011		2010	2011		2010	2011	
<i>T. viride</i>	1.0	1.59 (4.2)	3.17 (8.4)	2.38 (6.3)	17.47	17.71	17.59	6.94	7.19	7.07
<i>B. subtilis</i>	1.0	12.69 (20.22)	7.93 (15.8)	10.31 (18.01)	16.23	16.02	16.12	6.33	5.23	5.78
<i>A. chroococcum</i>	1.0	4.76 (12.6)	7.93 (15.8)	6.35 (14.2)	16.73	16.44	16.59	7.07	6.84	6.96
<i>T. harzianum</i>	1.0	17.45 (24.64)	20.63 (26.86)	19.04 (25.75)	16.48	16.44	16.46	6.64	6.30	6.47
<i>P. fluorescence</i>	1.0	19.04 (25.56)	28.57 (32.3)	23.81 (28.93)	14.33	12.57	13.45	3.60	2.55	3.07
<i>B. brevis</i>	1.0	23.81 (28.93)	28.57 (32.3)	26.19 (30.62)	12.25	12.49	12.37	2.19	2.05	2.12
Control	–	34.92 (36.2)	39.68 (39.02)	37.3 (37.61)	10.11	10.22	10.16	1.10	0.96	1.03
Mean		16.32 (21.76)	19.5 (24.35)		14.80	14.55		4.84	4.45	
CD _{0.05}		0.65			0.65			0.65		

* Figures in parentheses are arc sine transformed values

Table.4 Effect of dip treatment of unrooted carnation cuttings in botanicals and bio-pesticides on the incidence of *Fusarium* wilt and important plant growth parameters

Treatment	Conc. (%)	Diseases incidence (%)			Plant height (cm)			Root length (cm)		
		2010	2011	Mean	2010	2011	Mean	2010	2011	Mean
Neemajal (<i>Azadirachta indica</i>)	1.0	11.11 (19.37)	12.69 (20.78)	11.90 (20.08)	15.06	15.17	15.12	6.07	5.93	6.0
<i>Melia azedarach</i> (S) (Darek)	1.0	12.69 (20.78)	12.69 (20.78)	12.69 (20.78)	13.36	14.60	13.98	4.88	4.89	4.89
<i>Roylea elegans</i> (L) (Karu)	1.0	15.87 (23.41)	14.28 (22.19)	15.07 (22.8)	13.55	14.32	13.94	4.75	4.74	4.75
<i>Artemisia roxburghiana</i> (L) (Shambri)	1.0	19.04 (25.86)	19.04 (25.86)	19.04 (25.86)	13.16	13.10	13.13	3.32	2.75	3.04
<i>Cryptolepis buchanani</i> (L) (Dudhli)	1.0	19.04 (26.97)	19.04 (25.86)	19.04 (25.86)	13.15	12.99	13.07	2.92	2.34	2.63
<i>Ocimum sanctum</i> (L) (Tulsi)	1.0	20.63 (24.64)	20.63 (26.97)	20.63 (26.97)	12.63	12.95	12.79	2.89	2.87	2.88
<i>Eucalyptus globulus</i> (L) (Safeda)	1.0	17.45 (26.97)	19.04 (25.86)	18.25 (25.25)	13.26	13.37	13.32	3.3	3.31	3.3
<i>Aloe vera</i> (L) (Gharit kumari)	1.0	20.63 (30.2)	22.21 (28.08)	21.42 (27.53)	12.97	13.01	12.99	2.85	2.6	2.73
Vermiwash	1.0	25.47 (38.98)	26.98 (31.26)	26.23 (30.73)	12.48	12.85	12.67	2.33	2.47	2.4
Control	-	39.68 (38.98)	44.44 (41.77)	42.06 (40.38)	9.94	9.95	9.95	1.12	0.60	0.86
Mean		20.16 (26.3)	21.10 (26.94)		12.96	13.23		3.44	3.25	
CD _{0.05}		2.52			0.49			0.44		

S: Seed meal; L: Leaves

* Figures in parentheses are arc sine transformed values

Table.5 Effect of soil solarization with transparent polyethylene sheet (25µm thick) on soil temperature in the polyhouse

Treatment	Soil depth (cm)	Maximum soil temperature (°C) during 1 May-9 June (2011)	
		Average	Range
Solarized with transparent polyethylene mulch (25 µm thick)	5	41.0	35.00 - 45.00
	15	35.8	29.00 - 39.00
Unsolarized	5	32.7	27.00 - 37.00
	15	29.8	26.00 - 34.00

Table.6 Effect of integration of soil solarization with effective root dip treatment of cuttings, soil amendment and combination of AM fungi, *Azotobacter chroococcum* and *Trichoderma viride* on the incidence of *Fusarium* wilt and important plant growth characters

Treatment	Disease incidence (%)			Plant height (cm)			Number of days taken for 1 st flowering			
	S	US	Mean	S	US	Mean	S	US	Mean	
T₁ (Root dip of cuttings in Bavistin @ 0.1%)	6.67 (14.79)	17.33 (24.56)	12.00 (19.68)	79.00	72.44	75.72	138.33	140.67	139.5	
T₂ (Soil application of <i>T. viride</i> @ 10g /1 kg of FYM /m ²)	9.33 (17.7)	17.33 (24.56)	13.33 (21.13)	78.67	70.33	74.5	138.00	141.67	139.83	
T₃ (Root dip of cuttings in Neemajal @ 20%)	10.67 (18.98)	22.67 (28.4)	16.67 (23.69)	75.67	67.89	71.78	140.33	144.00	142.17	
T₄ (Soil amendment with Neemcake @1 Kg/m ²)	12.00 (20.26)	21.33 (27.47)	16.67 (23.87)	77.78	69.56	73.67	138.67	143.00	140.83	
T₅ (T ₄ + T ₂)	8.00 (16.42)	14.67 (22.47)	11.33 (19.44)	82.66	76.78	79.72	136.67	137.00	136.83	
T₆ (T ₄ + T ₁)	6.67 (14.79)	16.00 (23.57)	11.33 (19.18)	81.55	74.00	77.78	135.00	137.33	136.17	
T₇ (T ₄ +, T ₃)	8.00 (16.42)	16.00 (23.57)	12.00 (20.00)	80.83	73.67	77.25	137.33	139.00	138.17	
T₈ (Root inoculation of cuttings with AMUHF @ 5g/plant + AZUHF @ 5g/plant + Soil application of <i>T. viride</i> @10g/1kg of FYM/m ²)	5.33 (13.16)	13.33 (21.36)	9.33 (17.26)	83.78	74.89	79.34	133.67	136.00	134.83	
T₉ (T ₄ + T ₈)	2.67 (7.69)	13.33 (21.36)	8.00 (14.53)	89.11	80.78	84.94	132.00	134.67	133.33	
T₁₀ (T ₁ + T ₈)	2.67 (7.69)	10.67 (18.98)	6.67 (13.33)	89	82.22	85.61	132.00	134.00	133.00	
T₁₁ (T ₃ + T ₈)	4.00 (11.53)	12.00 (20.26)	8.00 (15.9)	86.55	81.55	84.05	132.67	135.33	134.00	
T₁₂ (T ₁ + T ₄ + T ₈)	1.33 (3.84)	9.33 (17.7)	5.33 (10.77)	94.78	86.44	90.61	130.00	131.6	130.83	
T₁₃ (T ₃ + T ₄ + T ₈)	2.67 (7.69)	9.33 (17.7)	6.00 (12.69)	91.67	82.78	87.22	132.00	133.67	132.83	
T₁₄ (Control)	21.33 (27.35)	46.67 (43.06)	34.00 (35.21)	71.43	62.78	67.11	146.33	153.33	149.83	
CD_{0.05}										
	Treatment	3.43			2.91			4.71		
	Treatment x Solarization	4.85			4.12			6.67		

S, Solarized; US, Unsolarized

* Figures in parentheses are arc sine transformed

AMUHF, consortium made from different isolates of AM fungi; AZUHF, isolate of *Azotobacter chroococcum*

Table.7 Effect of integration of soil solarization with effective root dip treatment of cuttings, soil amendment and combination of AM fungi, *Azotobacter chroococcum* and *Trichoderma viride* on the important quality parameter of the flowers

Treatment	Number of flowers per plant			Flower size (cm)			Length of flowering stem (cm)			
	S	US	Mean	S	US	Mean	S	US	Mean	
T₁ (Root dip of cuttings in Bavistin @ 0.1%),	3.44	3.11	3.28	6.19	6.08	6.14	71.89	65.44	68.67	
T₂ (Soil application of <i>T. viride</i> @ 10g/1 kg of FYM/m ²)	3.56	3.22	3.39	6.11	6.00	6.06	71.44	64.89	68.17	
T₃ (Root dip of cuttings in Neemajal @ 20%)	3.22	2.78	3.00	6.00	5.57	5.78	69.67	60.67	65.17	
T₄ (Soil amendment with Neemcake @ 1 kg/m ²)	3.33	3.00	3.17	6.00	5.73	5.87	71	63.44	67.22	
T₅ (T ₄ + T ₂)	3.89	3.56	3.72	6.3	6.2	6.25	75.56	69.56	72.56	
T₆ (T ₄ + T ₁)	3.78	3.33	3.56	6.22	6.17	6.2	75.22	67.44	71.33	
T₇ (T ₄ + T ₃)	3.68	3.33	3.51	6.36	6.25	6.31	73.89	67.33	70.61	
T₈ (Root inoculation of cuttings with AMUHF @ 5g/plant + AZUHF @ 5g/plant + Soil application of <i>T. viride</i> @ 10g/1 kg of FYM/m ²)	4.00	3.67	3.83	6.36	6.25	6.31	77.67	68.22	72.94	
T₉ (T ₄ + T ₈)	4.33	4.00	4.17	6.7	6.37	6.53	83	75.67	79.33	
T₁₀ (T ₁ + T ₈)	4.11	3.89	4.00	7.07	6.45	6.76	82.67	75.56	79.11	
T (T ₃ + T ₈)	4.00	3.78	3.89	6.55	6.37	6.46	79.89	74.78	77.33	
T₁₂ (T ₁ + T ₄ + T ₈)	4.67	4.11	4.39	7.42	6.95	7.18	88.29	80.11	84.2	
T₁₃ (T ₃ + T ₄ + T ₈)	4.44	4.00	4.22	7.36	6.78	7.07	84.89	76.78	80.83	
T₁₄ (Control)	3.00	2.33	2.67	5.83	5.33	5.58	64.89	56.11	60.50	
CD_{0.05}										
	Treatment	0.28			0.23					
	2.61									
	Treatment x Solarization	0.40			0.33					
	3.69									

S, Solarized; US, Unsolarized

* Figures in parentheses are arc sine transformed values

AMUHF, consortium made from different isolates of AM fungi; AZUHF, isolate of *Azotobacter chroococcum*

This treatment combination is also effective in improving important plant growth and quality parameters with increase of 50.97, 100.4, 39.2, 57.3, per cent in plant height, number of flowers per plant, flower size and length of flowering stem, respectively in comparison to unsolarized and unamended control. This treatment combination also resulted in early flowering by 23.3 days in comparison to unsolarized control.

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