Botanicals, biofumigants and antagonists application in managing stem rot disease caused by *Rhizoctonia solani* Kuhn in carnation

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ABSTRACT

The pathogen, *Rhizoctonia solani* Kuhn is a polyphagous fungus and has a vast potential of attacking a wide range of host plants causing host decay, damping –off, stem rot, crown rot ,stem canker, root rot, fruit decay and foliage diseases. The stem rot is an emerging devastating disease in carnation rating next in importance to Fusarium wilt with a seedling mortality of 37% under optimum climatic conditions. Because of high incidence and crop importance, the present study was carried out to manage the disease with application of different plant extracts (botanicals), biofumigant releasing crop residues and biocontrol agents both under *in vitro* and field conditions. Out of 11 botanicals including two commercial formulations of neem (neemgold, neemazil) tested at 5,10,20,30,40% and 1,2,3,4,5% concentrations respectively revealed that seed extracts of *Melia azedarach* and leaf extract of *Adhatoda vasica* showed maximum inhibition in mycelial growth within the range of 44.38 to 44.25% followed by *Murraya koenigii* (37.77%) and *Tagetes erecta* (37.62%).The commercial formulations of neem compared to other treatments were found statistically superior to inhibits mycelial growth. In general, higher concentration resulted in more inhibition of the *R. solani*, above 50 % inhibition was recorded at concentration of 40 per cent, maximum being in neemgold (60.53%) compared to 5% or 10%. Among different crop residues, cauliflower and cabbage were found most effective in reducing the incidence of carnation stem rot to 22.28 and 24.25% from 48.34% registered in control. The plant and flower characters such as plant height, stem length, no. of flowers/plant, flower size/plant and no. of days to first flowering get reduced to 142 compared to 157 days. The average colony forming unit of the pathogen reduced to $22.83x10^3$ from $58.12x10^3$ in one cropping season. Antagonists, *Trichoderma viride, T. harzianum* and *Bacillus subtilis* resulted into mycelial inhibition of 65.08, 63.70 and 55.05%. However in field the highest disease control of stem rot was achieved in integrated treatments when the rooted cuttings of carnation were pretreated with neemgold or neemazil for 20 minutes as dip treatment and planted in soil amended with *T. viride* and *T. harzianum* by giving disease control between 63-73%.

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INTRODUCTION

Carnation botanically known as *Dianthus caryophyllus* L. is one of the popular flowers in the floriculturist trade and possesses important aesthetic value to the human beings. It belongs to caryophyllaceae and is indigenous to the Mediterranean areas. The flower is cultivated worldwide and is famous for the cut flowers due to variegated petal's colour, bedding, pot, borders, edging and rock garden. There are a few commercial varieties seen in the market which are highly in demand throughout the year and the flower is placed in the 7^{th} rank in the world flower trade. India has immense potential for growing high quality carnation for export as well for meeting the home demand in various occasions because of its varied climatic conditions and diverse eco-geographical regions. In Himachal Pradesh it occupies an area of 39.72 ha out of which the 30 millions stems have been registered during 2008-2009 (Anonymous, 2009). However, the flower is susceptible to a number of diseases. The prevalence of warm humid climate from June to August in Himachal Pradesh favours the occurrence of stem rot which may lead to rotting of the underground stem portion followed by wilting and death of plants. Outbreak of the disease was first recorded in Solan district of Himachal Pradesh by Meeta and Mathur (1991).

The loss induced by the stem rot was manifold. It may go as high as 37 per cent under optimum climatic conditions (Chandel, 2001). The most frequently applied method for disease control is through the use of fungicides or other cultural practices. However the excessive toxicants had an adverse effect on the soil fertility, environment and resulted in the development of the resistant strains of the pathogens which are more difficult to control. Hence looking into the various implications on the environment, the present study was based on use of different control strategies employing botanicals, crop residues of biofumigant releasing crops and biological control agents to manage the stem rot of carnation.

MATERIAL AND METHODS

Evaluation of botanicals

Hot water extracts of nine plants species namely *Melia azedarach*, (Darek- seed extract) and leaf extracts of *Eucalyptus globules* (Safeda), *Roylea elegance* (Karu), *Vitex negundo* (Banna), *Adhatoda vasica (*Basuti), *Tagetes erecta* (Marigold), *Murraya koenigii* (Currypatta), *Mentha arvensis* (Pudina), *Cannabis sativa* (Bhang) and two commercial formulations for neem were evaluated at 3 and 5 % *in vitro* against *Rhizoctonia solani*. The fresh leaves and seeds (200g) of each plant species were washed with running water, grinded for 5 min. in blender by adding a small quantity of sterilized warm distilled water. After grinding, 200 mL of distilled water was added and homogenized in orbital shaker at 2000 rpm for half an hour to get 100 per cent extract of different plant parts. The plant material was then filtered through muslin cloth. Sterilization of the extract of different plants was carried out in an autoclave at 5psi pressure for one hour for three consecutive days.

The prepared extracts were evaluated by Poisoned Food Technique given by Falck (1907) at 5, 10, 20, 30 and 40 concentrations and two commercial formulations of neem were tested at 1,2,3,4 and 5 concentrations. Evaluation of extracts was done at 5 per cent concentration by incorporating 5 ml of extract of botanical (100%) in 95 ml sterilized (autoclaved at 1.05kg/cm^2 for 20 min.) double strength PDA (potato dextrose agar) medium, cooled and poured in the sterilized Petri plates under aseptic conditions. In the same way, the extracts of other desired concentrations were prepared. The Petri plates were inoculated with 4 mm dia. bits of 7 day-old culture of the stem rot pathogen and incubated at 27^{o}C in BOD incubator. Petri plates containing 50 ml sterilized double strength PDA medium added to 50 ml sterilized distilled water served as control. The experiment was laid out in CRD and each treatment was replicated thrice. The per cent inhibition in the mycelium growth of the fungus was calculated as per the formula of Vincent (1947) I=C-T/Cx100, where I=inhibition of mycelium growth (%), C= linear mycelium growth in control (mm) after 24 hrs and T= linear mycelium growth in treatment (mm) after 24hrs.

Effect of biofumigants (cruciferous) crop residues on disease and plant growth parameters and survival of pathogen and soil microflora

In order to evaluate the effect of biofumigant crop residues, six crops of Brassicacae family, *Brassica juncea* (Indian mustard), *Raphanus sativum* (Radish), *B. chinensis*(Chinese Sarson), *B. oleracea* var. *italica* (broccoli), *B.oleracea* var. *capitata* (cabbage), *B.oleracea* var. *botrysis* (cauliflower) were selected and chopped into small pieces. These were later mixed in fresh form in upper layer of formalin sterilized soil (5%) which was artificially inoculated with mass culture of stem rot pathogen (5g wt/wt). Thereafter carnation cuttings of variety 'William Smith' were planted in the earthen pots of 12 inches dia. in CRD with four replications. The data pertaining to disease incidence (%), different plant growth and flower parameters were recorded at 10 days of intervals. In order to find out the colony forming unit (c.f.u) of the pathogen, one gram of soil which was mixed with crucifer crop residues was dissolved in 100ml of autoclaved sterilized water and then serial dilution was followed upto 10^{-4} concentration (Johnson, 1957). One ml of water was pippetted out from each dilution in each Petri plate containing semi-selective medium (Gutierrez *et al*., 2001). Dilutions of $10^{-4,}$ 10^{-5} and 10^{-6} were used to record the microflora of the soil constituting fungi, actinomycetes and bacterial populations. The data on disease incidence (%) different growth parameters and c.f.u of microflora were enumerated and analyzed.

***In vitro* and field screening of biocontrol agents**

The antagonist activity of the native biocontol agents particularly Trichoderma species, *T. viride, T. harzianum, T. virens, T. koeinigii* and three bacterial strains *Bacillus subtilis, Pseudomonas fluorescens* and *Bacillus* spp. were determined against *Rhizoctonia solani*.Six mm dia. bits of each antagonistic fungi was taken from vigorously growing margins and were transferred aseptically to one side of solidified PDA medium in the Petri plates. On the opposite side, same dia. bits of *Rhizoctonia solani* were inoculated by dual culture technique (Huang and Hoes, 1976). The bacterial antagonists were streaked on one side using 48 hr old colonies of bacteria after placing the cultural bits of the test pathogen as per the streak plate method described by Utkhede and Rahe (1983) and incubated at 27^{o}C in BOD incubator. Control treatment was without fungal and bacterial antagonists. The experiment was laid out in CRD with three replications and the data so obtained was analyzed statistically and per cent inhibition in the growth of the pathogen was ascertained. Further the effective treatments were tried in field in randomized block design as dip and soil mix treatments. The data describing disease incidence (%), and different plant growth and flower characters were recorded at 10 days intervals, pooled and analyzed statistically as per the procedure described by Gomez and Gomez (1983).

Combined effect of effective antagonists and botanicals

In field conditions the integrated effect of the biocontrol agents and botanicals were evaluated to formulate the suitable eco-friendly strategy in managing the serious stem rot disease of carnation. Three biocontrol agents (i.e. *T.harzianum, T. viride, Bacillus* spp.) and two commercial botanicals (neemgold and neemazal) were considered and applied as dip, soil mix and soil drench treatments. The fungal antagonists were applied @100g along with FYM in 1m*1m sized beds in solid form as soil mix while bacteria and neem formulations were used as dip in liquid form (2%) prepared in sterilized distilled water by dipping the rooted cuttings in solution for 30 min. prior to planting. The neem formulations were also used later on first outbreak of the disease for two times and antagonists were mixed once in the soil after one and a half month of planting of cuttings since the disease usually appears after this period in the field which coincides with the environmental factors. The fungal antagonists were procured from the Department of Plant Pathology and bacteria from the Department of Basic Sciences of the Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P., India). After treatment, carnation cuttings of variety 'William Smith' were planted at a distance of 20x20 cm in 1x1 sq meter beds with 25 cuttings per bed. Beds without application of antagonists and botanicals served as control. The experiment was conducted in RBD along with three replications. The data related to disease incidence (%) was recorded and per cent disease control was calculated.

RESULTS

All the extracts of nine different botanicals including two commercial formulations of the neem at different concentrations resulted in inhibition of the mycelial growth of the pathogen compared to control (Table1). Seed extract of *Melia azedarach* and *Adhatoda vasica* were found most effective and showed significant superiority amongst all the natural botanicals with 44.38 and 44.25 per cent inhibition in the growth of fungus followed by *Murraya koenigii* and *Tagetes erecta*. The results of interaction revealed that at minimum of 5% concentration least inhibition was obtained in *Mentha arvensis* and *Eucalyptus globulus* while maximum inhibition was recorded in neemgold. More inhibition was obtained at 30 and 40 per cent concentrations than low concentrations at 5 per cent, indicating high inhibition with increase in concentration Murthi and Sirsi (1957) reported the pharmaceutical properties of *Melia azedarach* and neem against different fungi and bacteria. Parmar and Ketkar (1996) concluded from their study that Nimbicidine and Neemactine possessed a fungicidal activity against many soil borne pathogens. The plant extracts of neem leaves and garlic leaves were reported to be very effective in controlling *Fusarium, Rhizopus and Curvularia* spp. causing seed rot of mustard (Zaman *et al.,* 1997). Use of plant extract as an antifungal substance against

Table 1. Efficacy of botanicals against the stem rot pathogen (*Rhizoctonia solani*) under *in vitro.*

Botanicals	Per cent inhibition in mycelial growth at concentration (%)					
	5	10	20	30	40	Mean
Murraya koenigii (L) (Currypatta)	28.14	28.51	29.25	49.25	53.70	37.77
Cannabis sativa (L) (Bhang)	25.18	26.29	27.03	49.63	53.70	36.37
Eucalyptus globulus(L) (Safeda)	24.07	25.55	25.92	26.29	28.14	25.99
Tagetes erecta (L) (Marigold)	27.77	28.14	28.88	49.63	53.70	37.62
Melia azedarach (S) (Darek)	38.14	38.88	39.25	51.63	53.33	44.25
Vitex negundo(L) (Banna)	25.92	26.66	27.03	48.52	49.63	35.55
Roylea elegans (L) (Karu)	27.77	28.13	28.88	40.74	50.00	35.10
Adhatoda vasica (L) (Basuti)	38.96	39.63	40.37	50.37	52.59	44.38
Mentha arvensis (L) (Pudina)	24.07	25.18	25.55	25.92	31.48	26.44
Neemajal	52.96	54.44	55.18	55.18	56.66	54.88
Neemgold	53.70	54.07	57.77	57.77	60.55	56.77
Mean	31.90	31.97	32.24	43.46	44.46	

* S- Seeds; L- Leaves; ** Neemazal and Neemgold tested at 1, 2, 3, 4 and 5 per cent;
Botanical:1.10; Concentration: 0.71; Botanical x Concentration: 2.45

Rhizoctonia solani was observed by different researchers (Sulistyani, 2004; Suprapta, 2006). Antifungal activity of different plant extracts have been observed against foliar pathogens by Verma *et al*. (2002) and Ali (2007). Mangang and Chhetry (2012) also showed efficacious action of *Coix lacryma jobi* and *Lantana camera* against mycelial inhibition of *Rhizoctonia solani*. The plant extracts when tried in pot and field conditions decreased the rot incidence significantly compared to control.

The results presented in Table 2 revealed that out of different crop residues, cauliflower was reported most effective in reducing the incidence of carnation stem rot to 22.28 per cent from 48.34 per cent obtained in control. Cabbage was found next in efficacy with disease incidence of 24.25 per cent while radish had the least effect in reducing the incidence of stem rot. Cauliflower also registered maximum average plant height, stem length, number of flowers per plant, flower size and required less number of days for first flowering compared to control where the plant had on an average significantly shorter plant height, stem length, less number of flowers per plant, flower size and took maximum number of days to first flowering. However, cauliflower, cabbage and broccoli responded equally effective with regards to appearance of first flowering. None of the treatments including control had any effect on calyx splitting. Average viable count of stem rot pathogen after addition of crucifer crop residues was reduced to 22.82×10^3 in comparison to control where average viability of the

Table 2. Effect of cruciferous crop residues on disease incidence, survival of stem rot pathogen and microflora of soil under pot conditions

Treatments	Disease incidence (%)	Plant height (cm)	Stem length (cm)	No. of days taken for 1st flowering	No. of flowers /plant	Flower size (cm)	c.f.u. g^{-1}
Radish	34.24	56.60	50.47	154.7	3.17	5.75	39.32×103
Cabbage	24.25	61.33	56.20	142.3	3.60	6.13	26.43×10^3
Broccoli	28.24	60.90	54.93	144.0	3.33	6.14	38.23×10^3
Chinese sarson	32.54	57.47	51.53	156.0	3.07	5.35	41.56×10^3
Cauliflower	22.28	62.30	56.20	142.7	3.70	6.37	22.82×10^3
Indian mustard	32.37	57.13	50.67	147.7	3.07	5.75	40.23×10^3
Control	48.34	41.47	35.27	157.7	2.61	5.24	58.12×10^3
CD (P=0.05)	3.20	1.04	1.93	1.97	0.46	0.36	0.07

pathogen remained 58.12x 10^3, estimated in per gram of soil. Gamliell and Stapleton (1993) also studied the release of volatile compounds in soil amended with cabbage residues and reported the inhibitory effect of such compounds on soil borne fungi like *Pythium ultimum* and *Sclerotium rolfsii*. Volatile compounds like phenyl isothiocyanate, allyl isothiocyanate, dimethyl sulphide, acitic acid, ethanol etc. were reported to be evolved from the leaf residues of the crucifer crops. Stapleton and Ducan (1998) also studied the effect of the cruciferous residues on the *Pythium ultimum*. Burgie (2005) found good efficacy of different Brassicaceae plants on damping-off of cucumber caused by *Rhizoctonia solani*.

Biological control agents also play an important role in the management of the soil borne pathogens and can become an important component in the integrated management strategy against the soil borne pathogens. In the present study five species of *Trichoderma* and two bacterial antagonists i.e. *Bacillus subtilis* and *Pseudomonas fluorescens* were evaluated under *in vitro* conditions (Table3). All the antagonists exhibited their inhibitory effect against test pathogen, but *T. viride* was found most efficacious with 65.08 per cent inhibition followed by *T. harzianum* (63.70 %). Out of two bacterial antagonists, *Bacillus* spp. and *B. subtilis* were found better than *Pseudomonas fluorescens* in inhibiting the mycelial growth upto 60.50 per cent. Rest of the

Table 3. *In vitro* screening of antagonists against *Rhizoctonia solani.*

Antagonists	Per cent inhibition in mycelium growth
Trichoderma harzianum	65.08
Trichoderma viride	63.70
Trichoderma koenigii	58.63
Trichoderma virens	54.56
Bacillus spp.	60.50
Bacillus subtilis	55.05
Pseudomonas fluorescens	53.25
CD (P=0.05)	0.86

antagonists was less effective but had a positive effect on inhibition of the fungus in comparison to control. *In vitro* screening for antagonism against *F. oxysporum* revealed significant inhibitory effects on mycelial radial growth to the tune of 3.25, 0.22 and 0.21 cm in well diffusion, streak and point inoculation method, respectively by Shobha and Kumudini (2012).

Antagonists that showed maximum inhibition of fungus when tested under laboratory were further evaluated in field conditions, revealed the positive

Table 4. Effect of antagonists on incidence of stem rot, plant growth and flower parameters of carnation

Antagonists	Conc. (%)	Disease incidence (%)	Plant height (cm)	Stem length (cm)	No. of days taken for 1st flowering	No. of flowers /plant	Flower size (cm)
Trichoderma viride	1%	17.33	71.80	65.57	133.20	3.53	6.24
T. koenigii	1%	26.67	67.20	64.27	133.20	3.47	6.21
T. harzianum	1%	20.00	69.40	60.60	137.60	3.27	5.82
T. virens	1%	37.33	62.80	56.46	143.05	2.87	5.65
Bacillus spp.	1%	28.00	66.88	59.28	137.80	3.20	5.76
Control		40.33	51.55	50.28	149.67	2.35	5.37
$CD_{(0.05)}$		3.70	5.27	5.21	10.79	0.72	0.37

The per cent disease incidence values are arc sine transformed

response on disease incidence and growth characters. Three antagonists *T. viride*, *T. harzianum* and *Bacillus* spp. reduced the disease incidence upto 17.33, 20.00 and 28.00 percent from 40.33 per cent registered in control as well as improved the plant growth with an increase of plant height (71.80 cm, 69.40 cm, 66.88 cm), Stem length (65.57 cm, 60.60 cm, 59.28 cm), number of flowers per plant (3.53, 3.27, 3.20), flower size (6.24 cm, 5.82 cm, 5.76 cm) and required least number of days (133.20, 137.60, 137.80) for first flowering (Table 4). Chet and Elad (1982) found that application of *T. harzianum* significantly reduced the incidence of *Rhizoctonia solani* and *Sclerotium rolfsii*. Chandel (2007) reported more than 60 per cent control in *Fusarium* wilt incidence of carnation by application of *T. harzianum* and *T. viride* in soil. Chakraborty and Chatterjee (2008) reported 86.44 per cent growth inhibition of *Fusarium solani* causing wilt of brinjal by *T. harzianum* under *in vitro* conditions. Inhibitory effect of different species of *Trichoderma* and *Bacillus* spp. have been reported by various workers against soil borne pathogens (Hatvani *et al*., 2006; Obiegilo, 1992; Kreb *et al*., 1993; Moon *et al*., 1995; Utkhede, 1993; Pandey and Upadhyay, 2000; Sharma, 2001; Khan and Khan, 2002; Saravanam *et al*., 2003; Ramesh and Korikanthimath, 2001). The data from field experiment of integrated study of botanicals and biocontrol agents presented in Table 5 revealed maximum disease reduction in case of neemgold treated plants as dip and soil amended with *T. harzianum* that gave 72.72 per cent control over disease with a minimum disease incidence of 8.0 per cent.

Table 5. Integrated effect of botanicals and biocontrol agents in managing stem rot

Antagonists	Disease incidence (%)	Disease control (%)
T. harzianum + neemgold	8.0	72.72
T. viride + neemgold	10.67	63.62
Bacillus spp. + neemgold	14.66	50.01
T.harzianum + neemazal	13.33	54.55
T. viride + neemazal	17.33	40.91
Bacillus spp.+ neemazal	12.00	\59.08
Control	29.33	--
CD (P=0.05)	0.28	--

Treatments of *T. viride* + neemgold and *Bacillus* spp.+ neemazil were next best in their efficacy followed by *Trichoderma harzianum* + neemazal compared to rest of the combinations in checking the disease incidence where the disease control was achieved within the range of 63 to 54 per cent. However, the later two treatments were statistically at par in their efficacy. Trillas *et al*. (2006) used composts from agricultural waste and the *Trichoderma asperellum*strain T-34 to suppress *Rhizoctonia solani* in cucumber seedlings while Ngullie and Daiho (2013) recorded reduced incidence of seedling rot in both greenhouse and field condition and high test per plant yield from

combination of *T. viride* + *P. fluorescens* followed by *T. viride.*

Thus it is predicted from the present study the possible use of *Trichoderma harzianum, T. viride* along with neemgold or neemazal and cruciferous residues particularly of cauliflower and cabbage (2g/kg of soil) can be a good alternative source to chemicals in managing stem rot of carnation in integrated form with minimum impact on the soil and environment.

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