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In vitro evaluation of botanicals and bio-pesticides against the *Fusarium* wilt of carnation (*Fusarium oxysporum* f. sp. *dianthi*)

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Abstract

Carnation wilt disease caused by *Fusarium oxysporum* f. sp. *dianthi* is the most serious disease of the carnation crop that restricts flower yield worldwide. In the present investigation, nine different bio-formulations were evaluated under *in vitro* conditions for their efficacy against the wilt pathogen and among these, Neemazal was found most effective and significantly superior amongst all the treatments with 73.06 per cent inhibition in mycelial growth of the wilt pathogen followed by extract of *Eucalyptus globulus* and *Melia azedarach* with 44.91 and 32.13 per cent inhibition in mycelial growth, respectively while the leaves extract of *Aloe vera* was found least effective with mycelial growth inhibition of 2.52 per cent only.

Keywords: Evaluation, botanicals, bio-pesticides, *Fusarium oxysporum*

Introduction

Carnation (*Dianthus caryophyllus* L.) is an important commercial cut flower of the world and it belongs to the family Caryophyllaceae. It is native to Mediterranean region. In this crop, there is good potential of getting high returns due to varied forms of flowers, different colour combinations and long vase life. Production and quality of flowers in carnation is affected by many biotic and abiotic factors and among these, diseases have been identified as major limiting factor. Among various diseases of carnation, *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *dianthi* is most prevalent and cause huge losses to the crop worldwide. The main problem in the management of *Fusarium* wilt is the endophytic nature of the pathogen and its persistence in the soil (Alstrom, 2001) [1]. Attempts have been made to control the pathogen by drenching the affected field with chemical fungicides (Biswas *et al.*, 2004) [3]. However, the continuous use of fungicides adversely affects the soil eco-system. Chemical fungicides prove lethal to beneficial soil micro flora including vesicular-arbuscular mycorrhizal fungi and also add to environmental pollution. Numerous strategies have been proposed and adopted to control this disease like use of antagonistic fungi (Manka *et al.*, 1997) [12], soil amendment (Hortink and Fahy, 1986) [10], Neem formulations (Bhat and Srivastava, 2003) [2], induced resistance (El-Khallal, 2007) [6] and by drenching the affected field with chemical fungicides (Biswas *et al.*, 2004) [3] etc. However, Botanical pesticides are considered as one of the eco-safe alternatives due to their biodegradation in nature, multiple mode of action on target pests and may not leave toxic residues. Botanicals are future potential sources for development of eco friendly products for crop protection.

Materials and Method

Hot water extracts of plants namely darek (*Melia azedarach* L.), karu (*Roylea elegans* Wall.), dudhli (*Cryptolepis buchanani* Roem. & Schult.), tulsi (*Ocimum sanctum* L.) shambri (*Artemisia roxburghiana*), safeda (*Eucalyptus globulus*), gharit kumari (*Aloe vera*), vermiwash and commercial formulation of neem (Neemazal 1.0 % EC) were evaluated under *in vitro* conditions against the test pathogen.

Preparation of plant extracts

Fresh leaves of karu, dudhli, tulsi, shambri, safeda and gharit kumari, seeds of darek, 200 g of each were taken and then washed under tap water and grinded for 5 minutes in blender by adding small quantity of distilled water. After grinding, 200 ml distilled water was added and homogenized in orbital shaker at the rate of 2000 rpm for half an hour to get 100 per cent extract of different plant parts.

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The plant material was then filtered through double-layered muslin cloth. Sterilization of the extract of different plants was done in an autoclave at 5 p.s.i. pressure for one hour and then the extracts were kept in refrigerator for further use.

In vitro efficacy of botanicals against the wilt pathogen

Evaluation of botanical extracts was done by poisoned food technique. Botanical extracts were tested at 25, 35, 50 and 75 per cent concentrations. Evaluation of the extracts at 50 per cent concentration was done by incorporating 50 ml of extract of botanical (100%) in 50 ml sterilized (autoclaved at 1.05 kg/cm² for 20 minutes) double strength PDA medium. It was cooled and then poured in the sterilized Petri plates under aseptic conditions. The Petri plates were inoculated with 4 mm diameter bits of 7 days old culture of the wilt pathogen. The extracts were also evaluated at 25, 35 and 75 per cent concentrations. Petri plates containing 50 ml sterilized double strength PDA medium mixed with sterilized distilled water served as control for comparison. Each treatment was replicated thrice and plates were incubated at 27±1°C in BOD incubator. Inoculated plates were observed daily and the colony diameter of test pathogen was recorded till the control plates were full with mycelium of the test pathogen. The per cent inhibition due to different treatments in the mycelial growth of the pathogen was calculated according to formula given by Vincent (1947)^[18].

$$I = \frac{C-T}{C} \times 100$$

Where,

I - Per cent inhibition of mycelial growth

C - Linear mycelial growth in control (mm)

T - Linear mycelial growth in treatment (mm)

Vermiwash was also evaluated at 25, 35, 50 and 75 per cent concentrations. Neemazal was also tested at 5, 10 and 20 per cent concentrations under *in vitro* conditions using poisoned food technique.

Results and Discussion

Extracts of different botanicals viz., leaves of tulsi, karu, dudhli, safeda, shambri, gharit kumari; seeds of darek; commercial formulation of neem (Neemazal) and vermiwash were evaluated under *in vitro* conditions against the wilt pathogen except for Neemajal, all were tested at 25, 35, 50 and 75 per cent concentrations by poison food technique, Neemazal was tested at 1, 5, 10 and 20 per cent concentrations.

All the extracts inhibited the mycelial growth of the pathogen in comparison to control (Table 1). Neemazal was found most effective and significantly superior amongst all the treatments

with 73.06 per cent inhibition in mycelial growth of the wilt pathogen followed by extract of *Eucalyptus globulus* and *Melia azedarach* with 44.91 and 32.13 per cent inhibition in mycelial growth, respectively (Plate 1). Further, *Artemisia roxburghiana* inhibited the mycelial growth of the pathogen by 22.78 per cent followed by 19.4 per cent in leaves extract of karu. Leaves extract of *Aloe vera* was found least effective with mycelial growth inhibition of 2.52 per cent only. As the concentration increased from 25 to 75 per cent, there was corresponding increase in per cent mycelial growth inhibition of the pathogen. Generally, a positive co-relation observed between concentrations of the tested biopesticides and inhibition of wilt pathogen (*Fusarium oxysporum* f. sp. *dianthi*).

There are several studies which highlight the efficacy of neem, other botanicals and other bio-resources against *Fusarium oxysporum* infecting carnation and other crops. Neem formulations contain high content of bioactive constituents like azadirachtin limonoids, terpenoids and flavonoids which are having anti-fungal properties. Antimicrobial activities against plant pathogenic root fungi have been reported due to secretion of allelochemicals like allacin in the bulbs of *Allium sativum*, capsaicin in the fruits of *Capsicum annum*, triterpenoids and flavonoids in the leaves and seed of *Melia azedarach*, Sesquiterpenes and monoterpenes in the leaves of *Ocimum* spp. and citronellol and menthol in the leaves and oil of *Eucalyptus* spp. (Gahukar, 1995)^[7]. A number of plant species have been reported to possess natural substances that are toxic to several plant pathogenic fungi (Goussous *et al.* 2010)^[8].

Nimbidine and Neemactine have been reported to be fungistatic against many soil-borne pathogens (*F. oxysporum*, *R. solani*, *S. rolfsii* and *S. sclerotiorum*) (Parmar and Ketkar, 1996)^[15]. Singh *et al.* (2002)^[16] found that neem product-Amritguard (200 ppm), inhibited the growth of mycelium of *Fusarium udum* by 69.2 per cent under *in vitro* conditions. Bhat and Srivastava (2003)^[2] also observed the effectiveness of Nimbidine and Neemazal against *Fusarium* wilt of carnation. Seed extract of *Melia azedarach* was found most effective with 84.63 per cent average inhibition of carnation wilt pathogen (*Fusarium oxysporum* f.sp. *dianthi*) (Negi, 2009)^[14]. Tomar and Chandel (2006)^[17] reported that leaf extract of neem (75%) is most effective resulting in complete inhibition of mycelial growth of *Fusarium oxysporum* f.sp. *gladioli* under *in vitro* condition. Moslem and El-Kholic (2009)^[9] reported the antifungal properties of neem leaves and seed extracts against plant pathogenic fungi like *F. oxysporum*, *A. solani*, *R. solani* and *S. sclerotiorum* under *in vitro* condition. Several other researchers have also found effectiveness of botanical like neem extracts and neem based bio-pesticides against *Fusarium* sp. (Dwivedi and Shukla, 2000; Jatav and Mathur, 2005; Chakraborty *et al.* 2009; Hanaa *et al.* 2011)^[5, 11, 4, 9].

Table 1: *In vitro* efficacy of botanicals and bio-pesticides against the wilt pathogen (*F. oxysporum* f.sp. *dianthi*)

Treatment	Per cent inhibition in mycelial growth at different concentration (%)				
	25	35	50	75	Mean
<i>Eucalyptus globulus</i> (L) (Safeda)	26.85 (31.14)	32.41 (34.68)	42.96 (40.94)	77.41 (61.65)	44.91 (42.1)
<i>Melia azedarach</i> (S) (Darek)	14.63 (22.4)	25.92 (30.59)	42.78 (40.83)	45.18 (42.22)	32.13 (34.01)
<i>Roylea elegans</i> (L) (Karu)	8.52 (16.95)	14.26 (22.16)	15.18 (22.9)	39.63 (39.00)	19.4 (25.25)
<i>Artemisia roxburghiana</i> (L) (Shambri)	15.56 (23.2)	16.85 (24.22)	23.33 (28.87)	35.37 (36.48)	22.78 (28.19)
<i>Cryptolepsis buchanani</i> (L) (Dudhli)	0 (0)	0 (0)	5.7 (13.81)	12.04 (20.26)	4.44 (8.52)
<i>Ocimum sanctum</i> (L) (Tulsi)	0 (0)	2.41 (8.83)	2.96 (9.77)	5.74 (13.83)	2.78 (8.11)
Neemajal* (<i>Azadirachta indica</i>)	38.74 (38.47)	66.66 (54.73)	86.85 (68.76)	100 (89.96)	73.06 (62.98)
<i>Aloe vera</i> (L) (Gharit kumari)	0 (0)	0 (0)	5.28 (13.27)	4.82 (12.57)	2.52 (6.46)

Vermiwash	0 (0)	0 (0)	2.93 (9.8)	7.22 (15.56)	2.54 (6.34)
Mean	11.59 (14.68)	17.61 (19.47)	25.33 (27.66)	36.38 (36.83)	

S, Seeds; L, Leaves

* Neemazal tested at 1, 5, 10 and 20 per cent concentrations

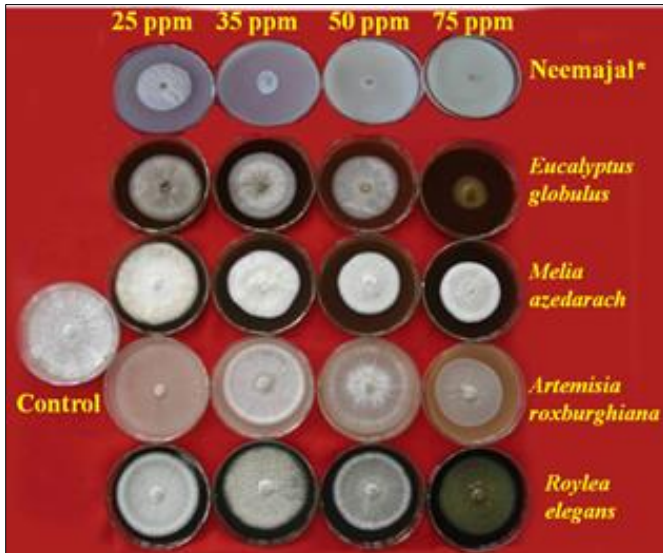
Figures in parentheses are arc sine transformed values

CD (0.05)

Treatment (1.06)

Concentration (0.41)

Treatment x Concentration (2.13)



* Neemazal tested at 1, 5, 10 and 20 per cent concentrations

Plate 1: Neemazal tested at 1, 5, 10 and 20

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