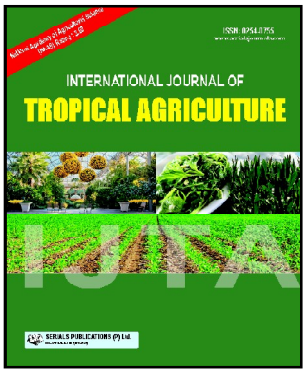

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Evaluation of botanicals against *Phytophthora cactorum* causing collar rot in apple

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Abstract: Botanical are ecologically safer, non-hazardous and non-polluting means of plant disease control. In the present investigations, fourteen botanicals were evaluated under *in vitro* and pot culture conditions against *Phytophthora cactorum* causing collar rot in apple. Water extract of mustard cake and *Eucalyptus* sp. leaves were the most effective and significantly superior amongst all the treatments exhibiting complete inhibition in mycelial growth of the pathogen. They were followed by karipatta leaves, mustard leaves and darek seeds extract with 78.01, 57.04 and 48.19 per cent mycelial growth inhibition, respectively. Among the five best *in vitro* evaluated botanicals tested under pot culture conditions, mustard cake water extract resulted in minimum disease incidence of 15.56 per cent followed by *Eucalyptus* leaves (20.0%) and were at par with each other. Extract of karipatta leaves was the next best treatment reducing the disease incidence to 31.11 per cent. Results also revealed that pre-inoculation of botanicals *i.e.* 7 days prior to pathogen inoculation, in general, reduced the incidence and severity of collar rot of apple significantly over their simultaneous application and after 7 days of pathogen inoculation. Minimum disease severity (7.33%) was also recorded with mustard cake followed by *Eucalyptus* leaves (9.29%) and these were significantly at par with each other.

Keywords: Apple, botanicals, extracts, *Phytophthora cactorum*

INTRODUCTION

Apple (*Malus* × *domestica* Borkh.) is one of the most important fruit crops generally grown in the temperate regions of the world. In India, it is commercially grown in the states of Jammu and Kashmir, Himachal Pradesh, Uttarakhand and parts

of Arunachal Pradesh, Nagaland and Sikkim (Chadha and Awasthi, 2005 [4]). Apple suffers heavily due to a variety of diseases which cause serious damage to the crop under favourable conditions, thus, affecting fruit quality and quantity. Of various diseases attacking apple, collar rot caused by *Phytophthora cactorum* is one of the most important soil-borne diseases, prevalent throughout the apple growing areas. The fungus attacks apple trees at soil level and girdles the stem/crown completely leading to death of the plant. Various fungicides have been recommended for management of the disease but soil drenching with fungicides is not cost effective. Further, continuous and non-judicious use of chemical fungicides in soil adversely affects the environment and soil microflora (Aktar *et al.*, 2009 [3]). More often chemicals fail to reach the infection site to eradicate pathogen (Utkhede, 1987 [9]) and sometime they act iatrogenically and aggravate the incidence of other soil-borne diseases. Botanicals are ecologically safer, non-hazardous and non-polluting means of disease management therefore, in the present investigation fourteen botanicals were evaluated under *in vitro* and pot culture conditions against *P. cactorum*.

MATERIALS AND METHODS

In vitro efficacy of botanicals

Water extracts of different plants *viz.,* leaves of *Murraya koengii* (karipatta), *Juglans regia* (walnut), *Melia azedarach* (darek), *Eucalyptus globulus* (eucalyptus), *Lantana camara* (lantana), *Artemisia roxburghiana* (artemisia), *Brassica campestris* (mustard), *Roylea elegans* (karu), *Vitex negundo* (bana) ; seeds of *Melia azedarach* (darek); cake of *Brassica campestris* (mustard), *Azadirachta indica* (neem) and *Gossypium hirsutum* (cotton); and cow urine were separately evaluated under *in vitro* conditions against the test pathogen at 10, 25, 50 and 75 per cent concentrations by poisoned food technique (Falck, 1907 [5]).

Preparation of plant extracts

Fresh leaves of karipatta, walnut, darek, *Eucalyptus*, *Lantana*, *Artemisia*, mustard, karu, seeds of darek, cake of mustard, neem and cotton weighing 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 extract of 100 per cent concentration. 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 psi pressure for one hour and then the extracts were kept in refrigerator for further use.

In vitro efficacy of botanicals against the pathogen

Evaluation of botanicals was done by poisoned food technique. Botanical extracts were tested at 10, 25, 50 and 75 per cent concentrations. Evaluation of the extracts was done at 50 per cent concentration by incorporating 50 ml of extract of botanical (100%) in 50 ml sterilized (autoclaved at 1.05 kg/cm^2 for 20 minutes) double strength Corn Meal Agar (CMA) medium, cooled and 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 pathogen. Similarly, the extracts were also evaluated at 10, 25 and 75 per cent concentrations. Petri plates containing 50 ml sterilized double strength CMA medium mixed with sterilized distilled water served as control for comparison. Each treatment was replicated thrice and plates were incubated at 22 ±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 [10]).

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

Where,

I - Per cent inhibition in mycelial growth

C - Linear mycelial growth in control (mm)

T - Linear mycelial growth in treatment (mm)

Similarly, fresh cow urine of local hill cow was taken and evaluated at 10, 25, 50 and 75 per cent concentrations.

Evaluation of botanicals under pot culture conditions

Water extracts of *Eucalyptus*, mustard cake, karipatta, mustard leaves and darek seeds which were five best botanicals under *in vitro* conditions were further tested under pot culture conditions by the method given by Gupta and Mir (1983 [6]) as below

Soil around the collar region was removed upto root zone. Polyethylene bags of 8 × 9 inch^2 size having both ends open were placed around the seedlings in a way to encircle them. Lower end of the polyethylene bags was pushed toward roots, tied with stem and the bag was inverted. This bag was filled back with sterilized soil, collar portion of the stem was given an injury by pinpricks and the bag further filled with soil upto above the collar region.

Different treatments of botanicals were added individually in soil inside bags @ 10 per cent simultaneously (at the time of inoculation) as well as 7 days prior and after the pathogen inoculation. The mass multiplied test pathogen was added in the soil @ 2 per cent (w/w). *P. cactorum* was mass multiplied on sand-maize-potato medium as follows:

The crushed maize grains were boiled just to soften them. Excess of water was drained out and maize grains were air-dried. Maize grains were then thoroughly mixed with sand @ 2 per cent along with 0.5 per cent sucrose and potato slices (2%). The medium thus prepared was filled in autoclavable polypropylene bags (1 Kg capacity), plugged with non-absorbent cotton and were sterilized by the process of tyndallization (autoclaving at 1.05 kg/cm^2 for one hour for two consecutive days). The sterilized medium was inoculated under aseptic conditions with 7 days old culture of *Phytophthora cactorum*. Five to six bits of 4 mm size of this fungus were placed in different sides of each bag. The bags were shaken regularly after 3 days so that the culture grows uniformly. The inoculated bags were incubated at 22 ±1°C in BOD incubator for 15 days and the mass culture was used for carrying out various experiments. The average c.f.u. recorded was 4.8 × 10^3/g of culture.

The data on disease incidence and severity was recorded upto 90 days after pathogen inoculation. Per cent disease incidence (PDI) was recorded as follows:

$$Disease\ incidence(\%) = \frac{Number\ of\ diseased\ plants}{Total\ number\ of\ plants\ observed} \times 100$$

Disease severity was recorded using 0-5 disease rating scale as described below:

Rating	*Collar area girdled (%)*
0	0.0
1	0.1-5
2	5.1-10
3	10.1-25
4	25.1-50
5	>50

The per cent disease Index was calculated according to McKinney (1923 [7])

$$Disease\ index\ (\%) = \frac{Sum\ of\ all\ disease\ ratings}{Total\ number\ of\ disease\ ratings \times Maximum\ disease\ grade} \times 100$$

RESULTS AND DISCUSSION

In vitro evaluation of botanicals

Water extracts of different plants part and cow urine were evaluated under *in vitro* conditions against the test pathogen at 10, 25, 50 and 75 per cent concentrations by poisoned food technique and it is evident from the data that all the extracts inhibited the mycelial growth of the pathogen in comparison to control (Table 1). Extract of mustard cake and *Eucalyptus* leaves were the most effective and significantly superior amongst all the treatments with 100 per cent inhibition in mycelial growth of the pathogen. They were followed by karipatta leaves and mustard leaves extract with 78.01 and 57.04 per cent mycelial growth inhibition, respectively. Extracts of leaves of walnut, karu and *Lantana* were least effective without any growth inhibition. Data also indicates that as the concentration increased from 10 to 75 per cent, there was corresponding increase in per cent mycelial growth inhibition of the pathogen.

Evaluation of botanicals under pot culture conditions

Extracts of *Eucalyptus*, mustard cake, karipatta, mustard leaves and darek seeds which were most effective under *in vitro* conditions were further tested under pot culture conditions.

The perusal of the data (Table 2) reveals that pre-inoculation of botanicals *i.e.* 7 days prior to pathogen inoculation, in general, reduced the incidence and severity of collar rot of apple

Table 1

In vitro* efficacy of botanicals against *P. cactorum

Treatment	*Per cent inhibition in mycelial growth at concentration (%)*				*Mean*
	10	*25*	*50*	*75*	
Melia azedarach seeds (Darek)	7.04 (2.83)	25.93 (5.19)	59.82 (7.79)	100 (10.05)	48.19 (6.47)
Melia azedarach (L)*	0 (1)	0 (1)	62.59 (7.97)	100 (10.05)	40.65 (5.01)
Murraya koengii (L) Kari patta	12.04 (3.56)	100 (10.05)	100 (10.05)	100 (10.05)	78.01 (8.43)
Juglans regia (L) Walnut	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)
Mustard cake	100 (10.05)	100 (10.05)	100 (10.05)	100 (10.05)	100 (10.05)
Vitex negundo (L) Bana	0 (1)	0 (1)	0 (1)	5.19 (2.49)	1.29 (1.37)
Eucalyptus globulus (L)	100 (10.05)	100 (10.05)	100 (10.05)	100 (10.05)	100 (10.05)
Cow urine	0 (1)	0 (1)	49.26 (7.09)	59.82 (7.79)	27.27 (4.22)
Lantana camara (L)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)
Neem cake	0 (1)	0 (1)	63.71 (8.04)	73.52 (8.63)	34.31 (4.67)
Brassica campestris (L) Mustard	0 (1)	28.15 (5.38)	100 (10.05)	100 (10.05)	57.04 (6.62)
Cotton cake	0 (1)	0 (1)	0 (1)	29.44 (5.52)	7.36 (2.13)
Artemisia roxburghiana (L)	0 (1)	0 (1)	42.96 (6.63)	100 (10.05)	35.74 (4.67)
Roylea elegans (L) Karu	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)
Mean	15.65 (2.61)	25.29 (3.55)	48.45 (5.91)	61.99 (6.98)	

*L- Leaves

Figures in parentheses are square root transformed values

$CD_{0.05}$	
Treatment	0.11
Concentration	0.06
Treatment × Concentration	0.23

significantly over their simultaneous application and after 7 days of pathogen inoculation. However, minimum disease incidence (15.56%) was recorded with mustard cake followed by *Eucalyptus* leaves (20.0%) and were at par with each other. Addition of mustard cakes has been reported effective against the collar rot (*P. cactorum*) and root rot (*Dematophora necatrix*) pathogens of apple (Sharma and Negi, 2013 [8]). Extract of karipatta was found next best treatment reducing the disease incidence to 31.11 per cent. Extract of mustard leaves had least effect on disease control with 54.0 per cent disease incidence. Minimum disease severity (7.33%) was also recorded with mustard cake followed by *Eucalyptus* leaves (9.29%) and these were significantly at par with each other. The inhibitory effect of mustard water extract could be attributed to the volatile compounds like butyl- isothiocynate, benzaldehyde, phenylacetaldehyde, myrcene and enzymes like protein kinase C, glycerate kinase and isozymes of nitrate reductase present in it (Anonymous, 2000 [1]). Leaves of *Eucalyptus globulus* contains protocatechuic, caffeic, gallic and oleanolic acids apart from volatile terpenes which could be the probable reason of their inhibitory effect on the test pathogen (Anonymous, 2002 [2]). Mustard leaves water extract also had least effect on disease control providing disease severity up to an extent of 37.86 per cent.

Table 2
Evaluation of botanicals under pot culture conditions

Treatment	*Disease Incidence*			*Over all Mean*	*Disease Severity*			*Over all Mean*
	-7days	*0 days*	*+7 days*		*-7days*	*0 days*	*+7 days*	
Murraya koengii (L)* (kari patta)	20.0 (26.35)	26.67 (30.96)	46.67 (42.68)	31.11 (33.3)	7.23 (15.56)	11.33 (19.66)	24.35 (29.56)	14.30 (21.59)
Eucalyptus globulus (L)	13.33 (17.70)	20.0 (26.35)	26.67 (30.96)	20.0 (25.0)	5.56 (13.62)	9.57 (18.01)	12.75 (20.84)	9.29 (17.49)
Melia azedarach seeds (darek)	26.67 (31.08)	33.33 (35.18)	46.67 (43.02)	35.56 (36.43)	7.83 (16.07)	16.32 (23.55)	25.47 (30.24)	16.54 (23.29)
Mustard cake	13.33 (20.97)	13.33 (17.70)	20.0 (26.44)	15.56 (21.71)	4.87 (12.59)	5.79 (13.36)	11.33 (19.64)	7.33 (15.19)
Brassica campestris (L) (mustard)	40.0 (38.84)	53.33 (46.90)	68.67 (56.16)	54.0 (47.30)	21.62 (27.69)	33.33 (35.23)	58.63 (50.0)	37.86 (37.64)
Control	85.0 (67.85)	82.89 (65.61)	83.67 (67.27)	83.85 (66.91)	76.67 (61.25)	78.89 (62.91)	77.78 (62.08)	77.78 (62.06)
Mean	33.06 (33.80)	38.26 (37.12)	48.72 (44.42)		20.63 (24.46)	25.87 (28.79)	35.05 (35.38)	

*L- Leaves

Figures in parentheses are angular transformed values

$CD_{0.05}$

Treatment	7.981	Treatment	3.144
Interval	5.643	Interval	2.223
Treatment × Interval	NS	Treatment × Interval	5.446

Application of botanicals 7 days prior to the pathogen application resulted in least disease incidence and severity. Presence of most effective botanicals have made the soil toxic thereby resulting in maximum disease control than simultaneous inoculation and inoculation of treatments seven days after the pathogen inoculation. Mustard cake and *Eucalyptus* leaves extracts were the best treatments with only 13.33 per cent disease incidence in both the cases when applied one week to the pathogen application. Mustard cake also resulted in least disease severity of 4.87 per cent when applied 7 days prior to the pathogen inoculation.

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