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Evaluation of Bioactive Plant Products for Ecofriendly and Effective Management of *Cercospora* Leaf Spot of *Rauwolfia serpentina* (L.) Benth Ex Kurz (Sarpagandha)

Pankaj Rautela, Vishwanath and Supriya Gupta
Department of Plant Pathology, College of Agriculture,
G.B. Pant University of Agriculture and Technology, Pantnagar, 263145, Uttarakhand, India

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Corresponding Author:
Pankaj Rautela
Department of Plant Pathology,
College of Agriculture,
G.B. Pant University of Agriculture and Technology, Pantnagar, 263145,
Uttarakhand, India

ABSTRACT
Rauwolfia serpentina (Indian snakeroot) is rich source of alkaloids, utilized for their medicinal properties. Diseases caused by foliar pathogens are major constraint in the economic production of the alkaloids from the dry roots of the plant. Among these diseases leaf spot caused by *Cercospora rauwolfiae* is of major importance, for its destructive nature resulting in huge economic losses. Management of the disease is achieved by use of chemical fungicides resulting in environment degradation and residual effect on the crop. Therefore in this study an attempt was made to evaluate the efficacy of commonly available essential oils and plant extracts for management of this destructive disease. Results of this study suggest that among the essential oils, mentha and peppermint oils at concentrations of 8 μL L^{-1} result in complete (100%) growth inhibition of the *C. rauwolfiae* while palmarosa oil was least effective in inhibiting *in vitro* growth (41.17%) of the fungus. In case of plant extracts garlic bulb extract was found to be most effective resulting in complete (100%) growth inhibition of the pathogen followed by neem, eucalyptus and turmeric respectively while leaf extract of the Ocimum gave minimum (17.25%) growth inhibition of the test fungus. Results of this study indicate that ecofriendly and effective management of *Cercospora* leaf spot of *Rauwolfia serpentina* can be achieved by using the metabolites (crude extract and essential oil) of plant origin.

Key words: *Rauwolfia serpentina*, plant extracts, essential oils

INTRODUCTION

Rauwolfia serpentina is affected by a number of foliar diseases caused by *Alternaria alternata*, *Cercospora rauwolfiae*, *Colletotrichum capsici*, *Coryneospora cassicola* and *Rhizoctonia solani*, which cause considerable damage to plants (Mohanthy and Addy, 1957; Ganguly and Pandotra, 1962; Shukla *et al*., 2010; Debjani, 2011). Among these, *Cercospora* leaf spot, caused by *Cercospora rauwolfiae* is major disease in North Indian plains resulting in severe reduction in the production of healthy seeds and roots. Foliar infection results in the decrease in alkaloid and steroid contents and increase in phenolics and flavonoids (Parashurama and Shivanna, 2013). Since, alkaloids obtained from *R. serpentina* are therapeutically important compounds used in treating various diseases and disorders in humans, decrease in alkaloid content causes yield and economic losses. Further, this disease adversely affect plantation as severe infection leads to defoliataion, deteriorate general appearance of the plant, resulting in qualitative and quantitative losses. There are many reports on the control of the diseases caused by the pathogen, *Cercospora* on other hosts (Prashanth, 2004;

Pairashi, 2007) but the work on control of *Cercospora* leaf spot disease of sarpagandha is at nascent stage. At present disease is managed by spraying contact or systemic fungicides. Use of fungicides for management of this disease is uneconomical due to high cost of chemicals and poses environmental threats with long term continuous use. Biological screening of plant extracts was carried out throughout the world for the determination of their antifungal activity. Synthetic chemicals used to control plant diseases not only pollute the environment but are also harmful to human health. Because of environmental and economic considerations, plant scientists are involved to find the cheaper and more environmental friendly biocompounds for the control of plant diseases using different forms of botanicals (Khare and Shukla, 1998; Mothana and Lindequist, 2005). Contrary to the problems associated with the use of synthetic chemicals, botanicals are environmentally safe, indigenously available, easily accessible, non-phytotoxic, systemic ephemeral, readily biodegradable, relatively cost effective and hence constitute a suitable plant protection in the strategy of biological management of diseases (Khadar, 1999; Kuberan *et al*., 2012). Therefore, development of alternative strategies based on biological/natural products is desirable for management of this disease.

The present investigations were carried out to evaluate the efficacy of different plant extracts and essential oils obtained from commonly occurring plant species for efficient, effective and ecofriendly management of *Cercospora rauwolfiae*. The study highlights the usage of commonly found bioactive compounds for disease management of this important foliar disease of *R. serpentina* and opens avenues for further exploration of alternative strategies for management of *Cercospora* leaf spot.

MATERIALS AND METHODS

Freshly infected leaves of sarpagandha exhibiting typical symptoms of *Cercospora* leaf spots were collected from field grown plants from Medicinal Research and Development Centre (MRDC), G.B. Pant University of Agriculture and Technology, Pantnagar. The pathogen *Cercospora rauwolfia* was isolated from infected leaves exhibiting leaf spot symptoms under aseptic condition. *Cercospora* affected leaves were placed in moist chamber and incubated in BOD (26±1°C) for 72 h.

The sporulated lesions were observed under binocular microscope. The single conidia were picked through glass needle and transferred aseptically in to six different plates containing PDA using single spore technique .The plates were placed in an incubator for 7 days. Pure cultures were obtained and were maintained in PDA.

Screening of plant extracts against *Cercospora*: Efficacy of different plant extracts from commonly occurring plant species. at different concentrations was evaluated for inhibition of radial growth of *Cercospora rauwolfia*. Extracts of different plant species viz., neem, eucalyptus, ocimum, garlic and turmeric were tested for their antifungal activity against *Cercospora rauwolfia* causing leaf spot of sarpagandha by poisoned food technique. The extracts of different plant parts were prepared by cold water extraction method described by Shekhawat and Prasada (1971).

Aqueous extract (at 2 mL g^{-1} fresh weight) were prepared by crushing samples in distilled water. The extracts were filtered through double layer of muslin cloth and later by Whatmann No. 1 filter paper. These filtered extracts were taken in the study as stock solution and were further diluted with distilled water before use. Double strength concentrations of botanicals were prepared by dissolving 10, 20, 30 and 40 mL of plant extract in 90, 80, 70 and 60 mL of sterilized distilled water to obtain final concentrations of 5, 10, 15 and 20%, respectively.

The botanicals were added to double concentration, sterilized oat meal agar medium in a laminar air flow chamber, poured in sterilized Petri plates (90 mm) and inoculated with 5 mm disc of 7 days old culture. The Petri plates were incubated at 26±1°C to study the inhibitory effect of botanicals on the mycelial growth of *Cercospora rauwolfia*. The data on radial growth of the fungus was recorded by measuring the colony diameter at 24 h interval upto 168 h and then the growth rate as well as percent inhibition were also calculated by using the following formula (Mckinney, 1923):

$$\text{Inhibition (\%)} = \frac{C-T}{C} \times 100$$

Where:
C = Growth in control
T = Growth in treatment

Screening of essential oils against the test pathogen: Efficacy of five essential oils i.e., peppermint oil (*Mentha pipperata*), patchouli oil (*Pogostimon patchouli*), mentha oil (*Mentha citrata*), palmaroza oil (*Cymbopogon martini*) and geranium oil (*Pelargonium graveolens*) was evaluated at four concentrations viz., 2, 4, 6 and 8 μL L^{-1}, paper discs of 5 mm were sterilized by steam sterilization in an autoclave (Remi Laboratory Instruments) and the disc was put into sterilized petriplate and with the help of micropipette different conc. of oil was put on the disc i.e., 2, 4, 6 and 8 μL L^{-1} and fungal disc of 5 mm were put in the petri plate having oat meal agar media 4 cm apart from each other. Then the Petri plates in replication of three were incubated at 26±1°C for 7 days. The growth of pathogen was measured after 7 days of incubation.

Statistical analysis: The data was analyzed statistically at the Computer Centre of G.B. Pant University of Agriculture and

Technology, Pantnagar, using Completely Randomized Design (CRD) with three replications. The treatments were compared by the means of Critical Differences (CD) at 5% level of significance. Means were compared using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$, by SPSS 16.0.

RESULTS AND DISCUSSION

The antifungal activities of different botanicals were tested *in vitro* by poisoned food technique. The data on the radial growth of the fungus was recorded periodically at 24 h interval. Inhibition of the mycelial growth of the pathogen *C. rauwolfia* varied significantly between different plant extracts. The significant difference was observed in mycelial growth inhibition by same plant extract at different concentrations viz. 5.0, 10.0, 15.0 and 20.0% which is comparatively lesser than control. Garlic extract showed complete (100%) inhibition in mycelial growth of *C. rauwolfia* followed by eucalyptus leaves (34.90%) at 5% concentration. However neem , turmeric and ocimum extracts showed 31.76, 23.14 and 11.96% growth inhibition, respectively indicating presence of certain chemical substances in their tissues that favoured the growth of *C. rauwolfia*. The least inhibition was exhibited by *Ocimum* leaves (46.47%) followed by turmeric rhizome (51.56%) at 20% concentration. However, eucalyptus leaves at 15 and 20% concentrations caused 52.55 and 64.11% inhibition in mycelial growth followed by neem 43.52 and 64.12%, respectively (Table 1).

Progressive increase in percent inhibition in mycelial growth was recorded with a corresponding increase in concentration of botanicals maximum being at 20% concentration However, garlic bulb extract exhibited most effective plant extract for inhibition of mycelial growth of *C. rauwolfia* at all the concentrations. Effectiveness of garlic in present study is in conformity with the findings of Swamy (2010), who observed that garlic bulb, neem leaves, neem seed kernel and eucalyptus leaf extract at 10% concentration showed hundred percent inhibition of *Cercospora capsici*. Effectiveness of garlic extract as mycelial growth inhibitor of *C. rauwolfia* could be ascribed to the fungicidal action of more than 200 different chemical substances including allin, allicin, allitin serordine, scordine and sativin I and II (Nene and Thapliyal, 2000; Misra and Dixit, 1976).

Effect of essential oils on growth of the test fungus: The efficacy of five essential oils viz., patchouli oil (*Pogostimon patchouli*), geranium oil (*Pelargonium graveolens*), mentha oil (*Mentha citrata*), palmaroza oil (*Cymbopogon martini*) and peppermint oil (*Mentha pipperata*) tested against *C. rauwolfia* using poisoned food technique has been shown in Table 2. The result obtained in present experiment conducted showed that the inhibition of mycelial growth varied significantly with different essential oil at different concentrations viz., 2, 4 6 and 8 μL. The data revealed that maximum inhibition of mycelial growth was recorded in mentha oil (83.33%) followed by pepper mint (77.26%), geranium (34.90%) and palmarosa (24.70%) while minimum inhibition of mycelial growth was recorded in case of patchouli oil (23.92%) at concentration of 2 μL. Further the data suggests that mentha oil is most effective essential oil for

Table 1: Effect of plant extracts of different concentrations on radial growth of *Cercospora rauwolfia*

Plant species	Plant parts used	Concentration (%)	Average colony diameter (mm)*	Growth inhibition (%)*
Neem	Leaf	5	58.00±1.00^{c}	31.76±1.17^{c}
		10	54.67±0.57^{b}	35.68±0.67^{b}
		15	48.00±1.00^{c}	43.52±1.17^{a}
		20	45.50±0.50^{e}	64.12±0.58^{d}
Garlic	Bulb	5	0.00±0.00^{a}	100.00±0.00^{e}
		10	0.00±0.00^{a}	100.00±0.00^{c}
		15	0.00±0.00^{a}	100.00±0.00^{c}
		20	0.00±0.00^{a}	100.00±0.00^{d}
Eucalyptus	Leaf	5	55.33±1.15^{b}	34.90±1.35^{d}
		10	55.33±1.15^{b}	34.90±1.35^{b}
		15	40.33±2.08^{b}	52.55±2.44^{b}
		20	33.33±1.52^{c}	64.11±0.58^{c}
Ocimum	Leaf	5	74.83±0.76^{e}	11.96±0.89^{a}
		10	70.33±2.08^{c}	17.25±2.44^{a}
		15	48.33±1.15^{c}	43.14±1.35^{a}
		20	30.50±0.50^{b}	46.47±0.58^{a}
Turmeric	Rhizome	5	65.33±0.58^{d}	23.14±0.67^{b}
		10	55.83±1.75^{b}	34.32±2.06^{b}
		15	49.67±1.52^{c}	41.56±1.79^{a}
		20	41.17±0.76^{d}	51.56±0.89^{b}
Control			85.00±0.00	0.00±0.00
CD at 5%				
Botanicals (a)		0.82		
Concentrations (b)		0.67		
Interaction (axb)		1.65		

*Data is expressed as Mean±SD (n = 3), means within the same column and followed by same letter are not significantly different according to Duncan's multiple range test ($p \leq 0.05$)

Table 2: Efficacy of different essential oils on the growth of *Cercospora rauwolfia*

Essential oils	Concentration (µL)	Average colony diameter (mm)*	Growth inhibition (%)*
Patchouli	2	64.67±0.57^{d}	23.92±0.67^{a}
	4	60.67±0.57^{d}	28.62±0.67^{a}
	6	43.33±1.52^{c}	49.02±1.79^{c}
	8	35.33±0.57^{b}	58.43±0.67^{c}
Geranium	2	55.33±1.52^{c}	34.90±1.79^{b}
	4	49.00±1.00^{c}	42.35±1.17^{b}
	6	45.67±1.15^{d}	46.27±1.35^{b}
	8	43.76±0.57^{c}	48.50±0.80^{b}
Mentha	2	14.17±0.28^{a}	83.33±0.34^{d}
	4	12.33±0.57^{a}	85.49±0.67^{d}
	6	8.67±0.57^{a}	89.80±0.67^{e}
	8	0.00±0.00^{a}	100.00±0.00^{c}
Peppermint	2	19.33±0.57^{b}	77.26±0.67^{c}
	4	15.67±0.57^{b}	81.56±0.67^{c}
	6	12.67±0.57^{b}	85.09±0.67^{d}
	8	0.00±0.00^{a}	100.00±0.00^{c}
Palmarosa	2	64.00±1.00^{d}	24.70±1.17^{a}
	4	61.00±1.00^{d}	28.24±1.17^{a}
	6	54.67±0.57^{e}	35.68±0.67^{a}
	8	50.00±1.00^{d}	41.17±1.17^{a}
Control		85.00±0.00	0.00±0.00
CD at 5%			
Essential oils (a)	0.60		
Concentrations (b)	0.49		
Interaction (axb)	1.21		

*Data are expressed as Mean±SD (n = 3), means within the same column and followed by same letter are not significantly different according to Duncan's multiple range test ($p \leq 0.05$)

inhibition of the *Cercospora rauwolfiae*, at all concentrations tested followed by pepper mint oil showing 100% growth inhibition above 6 µL results also show that more than 50% inhibition was observed in patchouli oil, followed by geranium oil (48.43%) while, minimum inhibition in mycelial growth was recorded in palmarosa oil (41.18%) at 8 µL concentration. In case of essential oils it was found that most effective oils for the management of *C. rauwolfiae* can be obtained from mentha and pepper mint. Both of these oils proved to be best for *in vitro* growth inhibition of the test pathogen at 8 µL L^{-1} concentration and had maximum efficacy. Experiment conducted by different workers showed that higher concentration of some essential oils inhibits the mycelial growth of various fungi as reported by Kizil *et al*. (2005). The present study is in accordance with Bisht *et al*. (2013), they reported that maximum inhibition of fungal pathogen was in higher concentration of peppermint oil. According to the study of Farrag (2011), lemon grass oil was found at all concentrations had strong capacity to reduce the spread of CLS on Okra plant.

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