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## Antifungal activity of plant extracts against *Diplocarpon rosae* causing black spot of rose

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### Abstract

Rose (*Rosa* spp), one of the most commercially valued flower crops, is extensively cultivated all around the world. Black spot caused by *Diplocarpon rosae* Wolf. is a most devastating and widespread disease in rose plants. Due to its severe infection during rainy conditions repetitive application of chemical fungicides becomes necessary for its control. Frequent application of synthetic fungicides leads to deteriorated quality and fragrance of flowers and hence its market value gets reduced. To overcome this problem, a study have been conducted to evaluate the antifungal activity of plant extracts against Rose black spot disease causing pathogen *Diplocarpon rosae*. Aqueous extracts of *Coleus forskohlii*, *Lantana camera*, *Leucas martinicensis*, *Withania somnifera*, *Sargassum muticum*, *Ulva lactuca*, *Zingiber officinale*, *Allium sativum* and neem oil were screened against the pathogen by poisoned food technique at 10 and 20 per cent concentration. The result revealed that three plant extracts - *Allium sativum*, *Leucas martinicensis* and *Zingiber officinale* showed significant antifungal activity against *D.rosae*. According to the obtained result, these plant extracts can be used as biological fungicide against rose black spot disease at field.

**Keywords:** Black spot, rose, antifungal activity, plant extract

### Introduction

The rose has established itself as the most popular garden plant in the world as well as the most important commercial cut flower grown for aesthetic as well as commercial purposes throughout the world. However, its cultivation demands special care and like any other crop, it is also attacked by a number of fungal, bacterial and viral diseases. Black spot caused by *Diplocarpon rosae* Wolf. is the most serious disease of *Rosa* spp. at nursery as well as field (Horst *et al.*, 1992). Due to its aesthetic value, rose plants are used for landscaping and grown in gardens. The feathery black spots on the leaves, yellowing, defoliation and reduced quality of flowers leads to reduction of market value as well as landscape management of rose plants (Drewes-Alvarez 2003) [2]. Frequent application of synthetic fungicides to control the disease leads to environmental and human hazard. To overcome this, botanical extracts are being tested to control the plant phytopathogens (Jeliazkova *et al.*, 2012) [3]. Many scientists have reported that extracts of many higher plants show antifungal and antibacterial activity under laboratory experiments. Since, plant based products are highly degradable and non hazardous to environment and humans, plant extracts can be used as alternative to synthetic fungicides (Varma and Dubey 1999) [8].

The objective of the present study was to evaluate aqueous extracts from various plant species for inhibition and control of black spot causing pathogen *D.rosae*, which is severe problem in rose gardens.

### Materials and Methods

#### Surface sterilization of plant parts

Fresh and disease free leaves and bulbs in case of *Allium sativum* and rhizomes in case of *Zingiber officinale* were collected and washed in running tap water. Then they were surface sterilized with 0.1 per cent mercuric chloride for 30 seconds and then washed with sterile water thrice.

#### Extraction of aqueous plant extract

A known quantity of surface sterilized plant materials were individually macerated using sterile pestle and mortar with equal volume of sterile water. The macerated plant material was then filtered through sterile muslin cloth and the extract was centrifuged for 10 minutes at 5000 rpm. The supernatant was then filtered through Whatman No.1 filter paper and stored at 4<sup>o</sup> C.

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### Screening of antifungal activity of plant extracts by poisoned food technique

The ten and twenty ml extracts were mixed with 90 and 80 ml of sterile PDA medium to get ten and twenty per cent concentration respectively. Then twenty ml mixture was poured into sterilized Petri plates and allowed to solidify. Using sterile cork borer nine mm diameter fungal disc of *D. rosae* was cut from old culture and placed at the centre of the Petri plate containing solidified medium. Each treatment was replicated thrice. PDA medium without any plant extract was used as control. In case of neem oil, ten and twenty ml of neem oil was directly added to the medium. Medium without any plant extract inoculated with fungal disc was used as control. The inoculated plates were incubated at  $25 \pm 1^\circ\text{C}$  for seven days. The diameter of the mycelial growth was documented and the per cent inhibition was calculated as follows –

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

where,

I = Per cent inhibition

C = Fungal growth in control plate (mm)

T = Fungal growth in treatment plate (mm)

### Statistical Analysis

The recorded data in different experiments were analysed statistically by using SPSS (Statistical Package for the Social Sciences) version 15.0 and SAS (Statistical Analysis System). The data with percentage values were transformed into arcsine values and the Critical Differences were worked out at 5% significant levels. By using Duncan's Multiple Range Test (DMRT), the means were compared.

### Result

Evaluation of plant extracts against *D. rosae* under *in vitro* condition by poisoned food technique revealed that out of nine treatments, six treatments showed varying degree of inhibition percentage whereas three treatments (*Withania somnifera*, *Ulva lactuca* and *Sargassum muticum*) showed no inhibition at all (Table 1). At 10 per cent concentration, *Allium sativum* showed highest inhibition percentage (38.60%) followed by *Leucas martinicensis* (35.29%). At 20 per cent concentration, neem oil showed highest inhibition percentage (60.36%) followed by *Allium sativum* (54.77%), *Leucas martinicensis* (46.92%) and *Zingiber officinale* (41.85%).

**Table 1:** Antifungal activity of different plant extracts against *D. rosae*

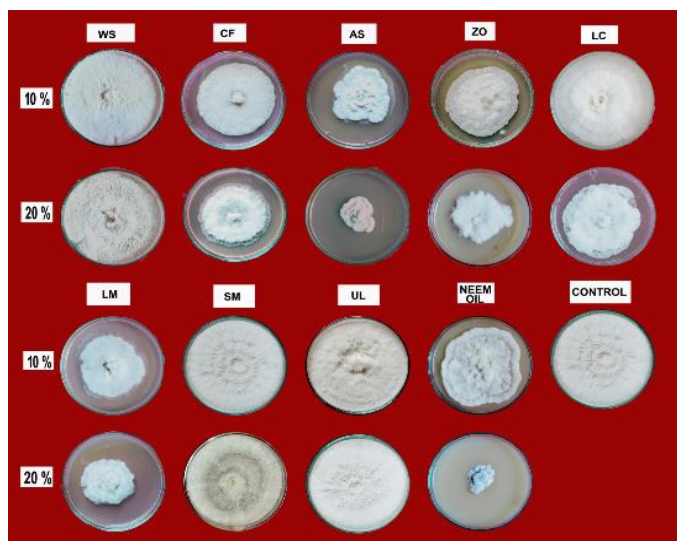
Treatment	Plant extract	*Mycelial growth at different concentration (in cm)		*Per cent mycelium inhibition over control at different concentration	
		10%	20%	10%	20%
T <sub>1</sub>	<i>Coleus forskohlii</i>	7.2	6.5	20.00 (26.58)c	27.78 (31.81)e
T <sub>2</sub>	<i>Latana camera</i>	8.5	7.7	5.56 (13.64)e	14.44 (22.34)f
T <sub>3</sub>	<i>Leucas martinicensis</i>	6.0	4.2	33.33 (35.29)b	53.33 (46.92)c
T <sub>4</sub>	<i>Withania somnifera</i>	9.0	9.0	0.00 (0.00)f	0.00 (0.00)g
T <sub>5</sub>	<i>Allium sativum</i>	5.5	3.0	38.89 (38.60)a	66.67 (54.77)b
T <sub>6</sub>	<i>Zingiber officinale</i>	7.3	5.0	18.89 (25.72)d	44.44 (41.85)d
T <sub>7</sub>	<i>Sargassum muticum</i>	9.0	9.0	0.00 (0.00)f	0.00 (0.00)g
T <sub>8</sub>	<i>Ulva lactuca</i>	9.0	9.0	0.00 (0.00)f	0.00 (0.00)g
T <sub>9</sub>	Neem oil	7.2	2.2	20.00 (26.66)f	75.56 (60.36)a
T <sub>9</sub>	Control	9.0	9.0	0.00	0.00
	CD (P=0.05)	0.039	0.047	0.154	0.056

\*Mean of five observations

The treatment means are compared using Duncan multiple range test (DMRT)

Values in parentheses are arcsine transformed

In a column, mean followed by a common letter (s) are not significantly different (p=0.05).



**Fig 1:** WS-*Withania somnifera*; CF-*Coleus forskohlii*; AS-*Allium sativum*; ZO-*Zingiber officinale*; LC-*Leucas martinicensis*; LM-*Leucas martinicensis*; SM-*Sargassum muticum*; UL-*Ulva lactuca*

### Discussion

The study suggests that neem oil, *Allium sativum*, *Leucas martinicensis* and *Zingiber officinale* can be used to control the black spot disease of rose. Similar research on antifungal activity of various plant extracts against plant pathogens have been reported by various researchers (Jeliazkova, (2012) [3]; Mohana (2007) [4]; Bhagwat and Datar, 2013) [1]. The antifungal activity of *Abrus precatorius*, *Aegle marmelos*, *Areca catechu* and *Brassica juncea* have been proven against *Aspergillus niger* (Raji and Raveendran, 2013) [6]. Aqueous extract of *Decalepis hamiltonii* showed significant antifungal activity against eight species of *Fusarium*, ten species of *Aspergillus*, three species of *Penicillium*, two species of *Drechslera* and *Alternaria alternata* (Mohana and Raveesha, 2007) [4]. Leaf extract of *Phytolacca americana* showed highest antifungal activity against *Botrytis cinerea* (Onaran *et al.*, 2016) [5]. Alcoholic extract of *Melia azedarach* actively inhibited the growth of various pathogens *viz.* *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum* and *Rhizopus stolonifer* (Sen and Batra, 2012) [7]. These findings suggest

that plant extracts can be used as ecofriendly alternative to chemical fungicides against plant pathogenic microbes.

### Conclusion

The findings of this study suggests that extracts of *Allium sativum*, *Leucas martinicensis* and *Zingiber officinale* can be used to control *D.rosae* at field condition. Since, neem oil will show phytotoxic effect on host plant at higher concentration, it could not be suggested for field application. It could be concluded that *Allium sativum*, *Leucas martinicensis* and *Zingiber officinale* can be potentially used for organic farming and as effective alternative for synthetic chemicals. Various biologically active molecules possessing antimicrobial properties are present in plant parts. These active compounds should be identified and produced in higher quantity for effective control of various phytopathogens.

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