Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; SP1: 278-280

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(Special Issue- 1)
2nd International Conference
"Food Security, Nutrition and Sustainable Agriculture - Emerging Technologies"
(February 14-16, 2019)

To study the effect of media on growth of the *Marssonia rosae*

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Abstract
Rose (*Rosa* sp.) belongs to family *Rosaceae*. Black spot, *Diplocarpon rosae*, is a fungal disease specific to roses. It is caused by a common fungus that thrives in wet, warm, and humid conditions. It is dominant and occurs worldwide and is the most severe disease of rose in almost all species and cultivars of Rose. The fungus infects leaves in the spring and reduces the plant's vigor throughout the growing season. In the present research, Cultural studies of *Marssonina rosae* were carried under in vitro conditions in order to find out the best liquid and solid medium for the vegetative growth and sporulation of the test fungus. Out of five solid media maximum radial growth was recorded on Malt Extract Agar (76.56mm) with sporulation count of 55.02 x 104 and out of five liquid media maximum dry mycelial weight (292.20mg) and sporulation of 24.46 x 104 spores/ml was observed on Richard's medium.

Keywords: Rose, black spot, media

Introduction
The rose is a widely studied plant and much has been written about it. Without delving much in to the history suffice it to say that the wild roses are believed to have their origins in Europe, America, the orient and China, and in the Middle East (Joyaux, 2003) [1]. Rose cultivation dates way back to around 3000 B.C. in the ancient Chinese gardens .Rose cultivars are commercially important. Every year 8 billion flowering stems, 80 million potted plants and 220 million garden rose plants are sold world-wide (Roberts *et al.*, 2003) [2]. It is also known as 'Queen of flowers'. Roses are symbols of love and beauty. Black spot disease is dominant and occurs worldwide and is the most severe disease of rose in almost all species and cultivars of Rosa in the outdoor (Horst, 1995) [3]. It is incited by *Diplocarpon rosae* (*anamorph Marssonina* rosae), an ascomycete fungus (Horst *et al.*, 2007) [4]. Though, it is a minor problem in greenhouse roses because humidity is regulated very carefully but is the most important disease of out door roses. The rose plant is used in gardens and landscaping for its aesthetic value, but the black spot infections make the roses unsightly due to the black spots on the leaves, yellowing and premature defoliation. Black spot disease manifests itself as circular black spots on plant foliage with an irregular margin on the upper surface of the leaf, which greatly reduce the beauty and performance of roses in the landscape. Black spot is easily distinguished from other diseases by the darker color and fringed borders of the spots that can occur on either side of the leaf. Spots often are surrounded by a yellow halo, and infected leaves fall prematurely. This disease may cause severe defoliation. As the lesions get increase in size, the leaves begin to yellow and abscise, compromising the health and appearance of the plant (Dobbs, 1984) [5]. The pathogen causes defoliation and reduced flower production and often results in weakening of the plants (Drewes-Alvarez, 2003) [6]. The premature defoliation leads to reduced vigour (Smith *et al.*, 1988) [7] and even death in very susceptible varieties (Black *et al.*, 1994) [8]. Disease damage cannot be assessed only in terms of size of lesion but always includes the defoliation aspect. The intensity and severity of black spot is quite high and reported to be 56, 42.14 and 56.00 per cent in different parts of the world (Wolf, 1912) [9]. However, during dry and cool months, plants remained infected throughout the year approximately by giving 30 per cent disease severity (Rangaswami *et al.*, 1970) [10].

The severity (S) and intensity (I) of *Marssonina rosae* in Tamil Nadu, Delhi, Solan (H.P.) and West Bengal were reported to be within a range of 14.1 to 30.0 per cent. Once established on plants, black spot is difficult to control despite a combination of practices that include sanitation measures and fungicide applications (Behe *et al.*, 1993) [11]. The conidia of the black spot pathogen germinate to form germ tubes on the host surface but further development of the fungus takes place below the host cuticle and even within the host cells. The control of this type of pathogen requires the use of contact as well as systemic fungicides (Gauchomo, 2005) [12].

Materials and methods

The present investigation studied in rose growing areas of two districts Solan and Sirmour of Himachal Pradesh were surveyed to record the black spot severity in rose. The areas surveyed include Nauni, Kandaghat, Mahog, Moginand, Sargaon, Matnali. At each location, thirty leaves selected from 4-5 plants were assessed randomly to record the disease severity on the basis of 0-6scale as adopted by Colbaugh *et al.* (2001) [13] with slight modifications. To observe the effect on mycelial growth and conidial production of the fungus different solid and liquid media were prepared (Appendix-I). In total 10 media, five each of solid and liquid medium namely Potato dextrose agar, Peptone potato dextrose agar, Malt extract agar, Glucose and asparagine agar, Rose leaf extract agar, Czapeck's Dox, Richard's medium, Brown's medium, Rose flower extract medium, Potato and carrot sucrose both were prepared, filtered through two layers of muslin cloth and sterilized by autoclaving at 15 lb psi pressure at 121ºC for 20 minutes. Growth and sporulation was enhanced with application of methionine in the medium @ 2 ppm as it acts as precursor of the ethylene which pathogen produces in plants. Sterilized Petriplates (90mm) and flasks (150ml) containing 25ml each of the above mentioned media were inoculated aseptically with uniform size bits (2mm) taken from the margin of an actively growing culture and incubated at 25 ±1ºC in BOD incubator. Each treatment was replicated five times. Data pertaining to mean radial growth and sporulation in case of solid media, and dry mycelial weight and sporulation in case of liquid media were recorded. The degree of sporulation was determined by the ratings given below using haemocytometer.

Spores (Conidia /ml) (10x or 40X)	**Degree of sporulation**
0	Absent
0-20	Poor
21-50	Good
>50	Excellent

Results and Discussion.

Cultural Studies

Cultural studies of *Marssonina rosae* were carried under in vitro conditions in order to find out the best liquid and solid medium, optimum temperature and pH for the vegetative growth and sporulation of the test fungus.

Effect of solid media

The results in Table 1. Revealed that all media supported for vegetative growth of the fungus, however, maximum growth and sporulation of *M. rosae* was found on malt extract agar medium (76.56 mm) after 15 days of incubation, which was followed by potato dextrose agar medium (54.49 mm), rose leaf extract agar medium (38.23 mm), peptone potato dextrose agar medium (28.90 mm). Glucose asparagine medium supported the least growth (20.50 mm) .With regard to degree of sporulation it was excellent in malt extract medium and potato dextrose medium, however in case of rose leaf extract and peptone potato dextrose agar media, the sporulation was good and rated poor in case of glucose and asparagines medium. Similar result was also recorded by Gauchomo (2005), the growth of fungal mycelium on malt extract agar could be seen with the naked eye from the 14th day of growth as small white points scattered on the surface of the growth medium. The top and the bottom of the fungal colony were white. Similar, growth pattern of fungal mycelia on potato dextrose agar (PDA) was observed by Leus (2005) [14].

Table 1: Effect of solid media on vegetative growth and sporulation of *M. rosae*

Media	**Mean radial growth (mm)**	**Sporulation**	
		Spores x 10^4 (conidia /ml)	**Degree of Sporulation***
Potato dextrose agar	54.49	52.05	Excellent
Rose leaf extract agar	38.23	25.46	Good
Peptone potato dextrose agar	28.90	28.74	Good
Malt extract agar	76.56	55.02	Excellent
Glucose and asparagines agar	20.50	14.33	Poor
$CD_{(0.05)}$	2.30		

* Degree of sporulation

Absent = 0, poor = 1-20, good = 20-50, excellent = > 50

Effect of liquid media

In liquid media studies, the perusal of results in Table 2. Revealed that maximum dry mycelial weight was recorded on the Richard's medium (292.20 mg), which was followed by Czapeck's Dox (251.00 mg), rose flower extract medium (242.60mg) and minimum was observed in Potato and carrot sucrose broth (30 mg).

Table 2: Effect of liquid media on vegetative growth and sporulation of *M. rosae*

Media	**Dry mycelia weight (mg)**	**Sporulation**	
		Spores x 10^4 (conidia/ml)	**Degree of Sporulation***
Czapeck's Dox	251.00	20.77	Good
Richard medium	292.20	24.46	Good
Brown medium	72.60	6.76	Poor
Rose(flower)extract medium	242.60	12.37	Poor
Potato and carrot sucrose broth	30.00	1.00	Poor
$CD_{(0.05)}$	4.59		

* Degree of sporulation

Absent = 0, poor = 1-20, good = 20-50, excellent = > 50

The degree of sporulation also varied which was good in both Czapeck's Dox and Richard's medium. However, poor sporulation was observed in rose flower extract medium, Brown's medium and Potato and carrot sucrose broth with spores less than 20 conidia/ml at 104 dilution. Effect of liquid media on mycelial growth and conidial production of *M.coronaria* was also studied by Zhao *et al.* 201016. Minimal salts medium was reported to support highest vegetative growth of *M. Brunnea* (Simpon and Hayes, 1978) [15].

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