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

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(4): 835-837
Received: 25-05-2020
Accepted: 27-06-2020

V Sai Deekshith
MSc Scholar, Uttaranchal University, Department of Plant Pathology, SOA, Dehradun, Uttarakhand, India

Jai Prakash Mishra
Principal & Professor, Department of Plant Pathology, Uttaranchal University, School of Agriculture, Dehradun, Uttarakhand, India

Rajendra Prasad
Asst-Professor, Department of Plant Pathology, Uttaranchal University, School of Agriculture, Dehradun, Uttarakhand, India

Corresponding Author:
V Sai Deekshith
MSc Scholar, Uttaranchal University, Department of Plant Pathology, SOA, Dehradun, Uttarakhand, India

In vitro cultural study of black spot of rose caused by *Diplocarpon rosea* against different solid media

V Sai Deekshith, Jai Prakash Mishra and Rajendra Prasad

Abstract
Black spot, the *Diplocarpon rosae* is a rose-specific fungal disease. It is caused by a common fungus, which thrives in humid, warm and wet conditions. This is prevalent and occurs across the world, and is the most serious rose disease in nearly all Rose species and cultivars. The fungus infects the leaves during the spring and reduces the vigor of the plant during the growing season. *In-vitro* cultural studies of *Diplocarpon rosea* were carried out of this five different solid media i.e. Sabouraud agar (SA), Potato dextrose agar (PDA), Czapek dox agar (CA),Corn meal agar (CMA), and Nutrient media (NA) of this PDA was recorded the maximum growth followed by Czapek dox agar (CA), and sabouraud agar (SA). where as nutrient agar recorded the minimum growth (NA).

Keywords: Cultural study, black spot, rose caused, *Diplocarpon* rosea, solid media

Introduction
Rose black spot is a fungal disease and the most serious rose-effected disease. This is caused by *Diplocarpon rosea*, a pathogen (F. A. WOLF 1912). On the upper surface of the leaves occurs the fungal disease Blackspot of roses is an important, devastating and widespread disease. In outdoor Black spot disease in the growth of roses is usually present, often as a major problem and Often with an epidemic proportion (Horst 1983) [3]. This is a slight greenhouse issue the rose plant is used for its landscaping and in gardens. Aesthetic value but infections with blackspot make the roses unsightly due to black Leaf streaks, yellowing and untimely defoliation. The cause of the pathogen Plant defoliation and weakening (Drewes-Alvarez 2003); The Untimely Defoliation leads to less vigor (Smith *et al* 1988) [8] and even very prone death Roses since humidity is very carefully controlled, but is the main disease of Roses outside the door (Horst, 1995).The harm D causes Rosae is larger than leaves Spots due to the added premature defoliation effect. Damage to the disease cannot be avoided Assessed only in terms of lesion size but also with the defoliation dimension. Blackspot, once established on plants, is difficult to monitor, despite a combination of Practices which include sanitation measures and applications of fungicides (Behe *et al* 1993) [2]. Healthy cultural practices include: removal and pruning of disease leaves from the soil Canes with infected leaves to develop pathogenic overwintering potential; Dense planting allowing good movement of air through the canopy of the leaves (Horst 1983) [3]; overhead irrigation as it promotes infection; prevent unnecessary watering During dark and humid weather; avoid long hours holding the rose leaves wet, as That provides the water needed to germinate the conidia.

Materials and Methods
Physiological studies on different solid media of *Diplocarpon rosea*
Five different solid media have been tested, namely, Sabouraud dextrose agar (SA), Potato dextrose agar (PDA), Czapek's dox agar (CA), Corn meal agar (CMA), Nutrient agar (NA). The best medium has been used for further maintenance, multiplication and selection of suitable physiological studies.

Different solid media
The following are the various solid media and their composition used in the process of this investigation.

Solid media

1.Potato Dextrose Agar (PDA) Medium

Peeled Potato Slices	200 gm
Dextrose	20 gm
Agar - agar	20 gm
Distilled water	1000 ml
pH	(6.0)

2.Czapek dox agar medium (CDA)

Potassium di hydrogen phosphate	1g
Sodium nitrate	3g
Potassium chloride	0.5g
Ferrous sulphate	0.01g
Agar agar	20g
Sucrose	30g
Mgso4	0.5g
Distilled agar	1000 ml
pH	7.3± 0.2

3.Sabourauds Dextrose Agar Medium (SDA)

Mixture of peptic digest of Animal tissues &pancreatic digest of casein (1:1)	10 gm
Dextrose	40 gm
Agar	15 g
Distilled Water	1000 ml
pH	5.6±0.2

4.Corn meal Agar (CMA)Medium

Corn meal, infusion from	50 gm
Agar	15 gm
Distilled Water	1000 ml
pH	(6.0)

5.Nutrient Agar medium (NA)

Peptic digest Animal tissue	5 gm
Sodium chloride	5 gm
Beef extract	1.5 gm
Yeast extract	1.5 gm
Agar	15 gm
Distilled water	1000 ml
pH	25 °C

Measure of growth

In order to assess the variability in the colony growth of *Diplocarpon rosea*, the colony growth of fungus was determined in each petri plate while the entire control plate was filled by fungus. The colony growth was measured at right angles between two diameters and averaged.

Study of colony and morphological characters

Observation of different cultural and morphological characteristics of mycelia viz., mycelial margin, growth pattern, mycelial color, mycelial growth disturbance, media distribution of all five different media were reported in each replication.

Result and Discussion

At the end of the seventh day of inoculation, the pathogen was grown on five different solid media selected for analysis. observations on *Diplocarpon rosea* 's radial growth rate were recorded. Regardless of the media used Potato dextrose agar (PDA) was found to be statistically higher than that of other cultural media followed by czapek dox and sabouraud agar. The nutrient agar (NA) has been found to be non-preferable culture medium for *Diplocarpon rosea* growth.

PDA was found to be the most suitable culture medium for the growth of *Diplocarpon rosea* with maximum radial growth rate. similarly, followed by czapek dox agar (CA) and sabouraud agar (SA). Where nutrient agar (NA) has been found to be non-preferable culture medium for the growth of *Diplocarpon rosea*.

Reetika *et al*. (2017) & Gauchomo (2005) also observed fungal growth patterns in various crop media such as potato dextrose agar (PDA), which were found to be the best medium for fungal multiplication.

Table 1: includes the morphological characters of the different solid media

Colony growth characters	PDA	CMA	SA	CZA	NA
Mycelial margin	Smooth	Smooth	Smooth	Filamentous	Dull
Growth pattern	Compact	Compact	Compact	Fluffy	Low
Mycelial colour	Light black	White	White	Pure white	Dull white
Distribution of mycelial growth	Thick	Thin	Thick	Thick	Irregular
Distribution of media	All over plate	All over plate	All over plate	All over plate	Periphery

Table 2: Radial growth (mm) of *Diplocarpon rosea* on different solid media

S. No.	Nutrient media	Radial growth of *Diplocarpon rosea*
1	Potato dextrose agar	70
2	Corn meal agar	45.5
3	Sabouraud agar	63
4	Czapek agar	67.5
5	Nutrient agar	6
	C.D.	1.111
	S.E. (m)	0.365

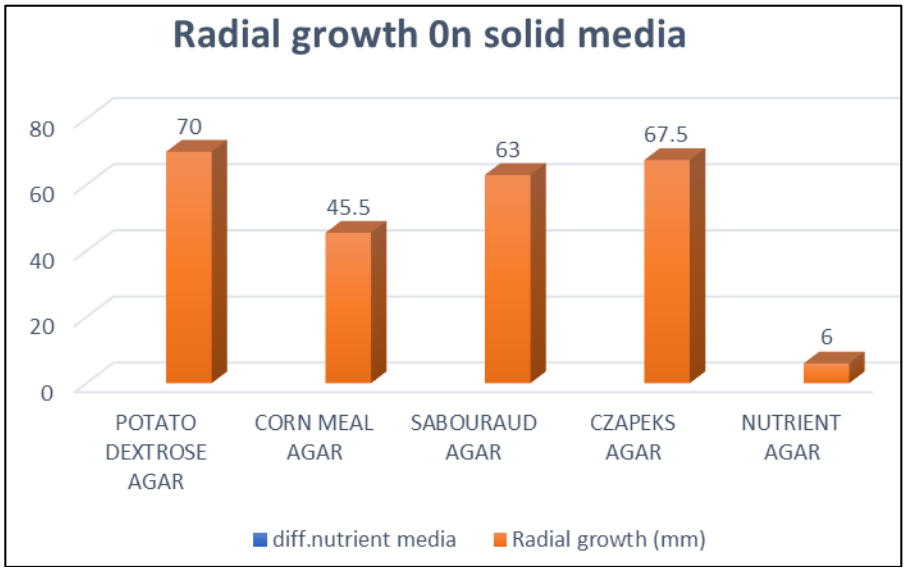
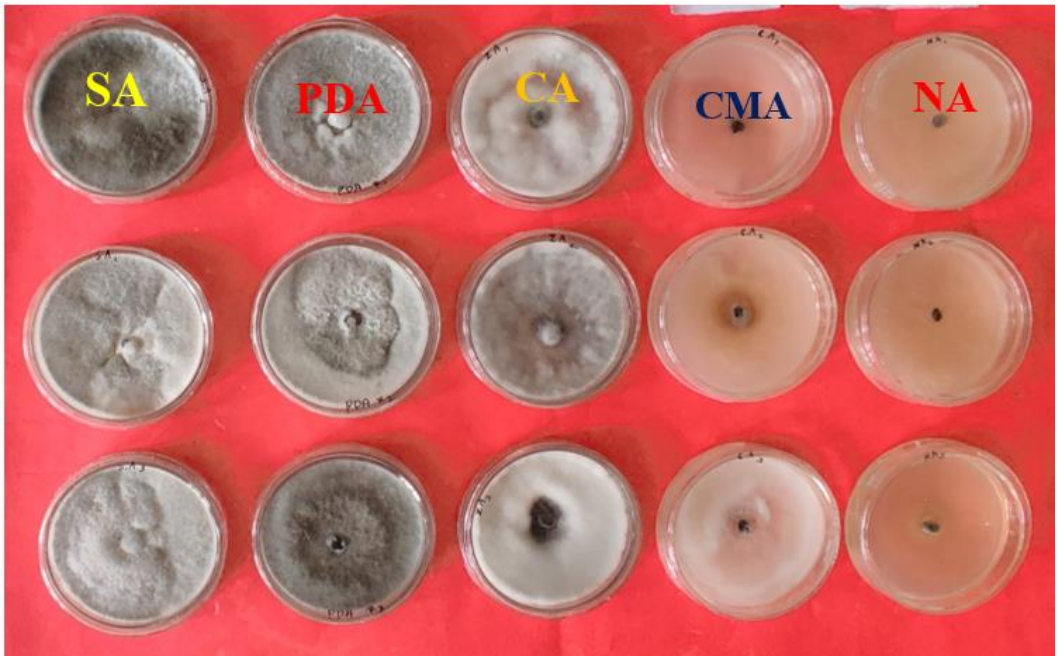


Fig 1: Graph shows that Radial growth (mm) of *Diplocarpon rosea* on different solid media



Pic 1: Includes effect of different solid media on the growth of mycelium on different media i.e. (SA, PDA, CA, CMA, NA.) of the pathogen *Diplocarpon rosea*

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