

FUNGAL DISEASES OF ROSE PLANT IN BANGLADESH

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ABSTRACT

Five types of symptom were recorded on two varieties of rose plant. The symptoms were Black spot, Leaf spot₁, Leaf spot₂, Blight and Anthracnose. The study revealed the presence of 20 species of fungi belonging to 17 genera. The isolated fungi were *Alternaria alternata* (Fr.) Keissler, *Arthrinium saccharicola* Stevenson, *Aspergillus flavus*, Link., *A. niger* van Tiegh., *Botrytis allii* Munn, *Cercospora* sp., *Cladosporium cladosporioides* (Fresen.) de Vries, *C. oxysporum* Berk. & Curt., two species of *Colletotrichum*, *Curvularia brakyospora* Boedijn, *Curvularia pallescens* Boedijn, *Fusarium* sp., *Epicoccum purpurascens* Ehrenb ex Schlecht; Link, *Gibberella* sp., *Marssonina rosea* (Lib.) Died, *Nigrospora sphaerica* (Sacc.) Masson, *Pestalotiopsis guepinii* (Desm.) Stay. with its two culture types, *Penicillium* sp., *Rhizopus stolonifer* (Ehrenb. Ex. Fr) Vuill. and *Trichoderma viride* Pers. ex Fries. The frequency (%) of association of *P. guepinii* was higher than any other fungi. *Pestalotiopsis guepinii* and its two culture types were found to be pathogenic to rose plant.

Key words: Fungal diseases, Rose plant

INTRODUCTION

Rosa centifolia L. and *R. involucrata* Roxb. belong to Rosaceae. It is a perennial shrub or vine of the genus *Rosa*. Plants are grown for their flowers' beauty and fragrance. The flowers are ancient symbol of love and beauty. Rose. has great value in perfume industry, Attar of rose is the steam-extracted essential oil from rose flower that has been used in perfumes for centuries. It has slightly astringent effect on the skin, muscles, tissues and cures eczema. Rose syrup is mostly made from an extract of rose petals. Rose hips are occasionally used in jam, jelly and marmalade for high vitamin C content. The hips are eaten by fruit eating buds. Its herbal tea is used in the treatment of cold and cough. The plant is affected by fungal disease along with bacteria, virus, nematodes and pests. The fungal diseases are Alternaria leaf spot, Black mold, Black spot, Botrytis blight, Brown canker, Cane blight, canker, Cercospora leaf spot, Common stem canker, Crown canker, Downy mildew, Fungal canker, Graft canker, Powdery mildew, Rust, Septoria leaf spot, Anthracnose and Verticillium wilt. The diseases are common throughout the world (Wikipedia 2013).

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Rose cultivation is now a profitable enterprise to the farmers, but the socioeconomic data and information of this flower are very scarce in Bangladesh. *Rosa* spp. are cultivated throughout the country as garden plant. It is commercially grown in Dhaka, Savar and Jessore (Haque *et al.* 2013). Lot of work has been done on fungal diseases of rose plant abroad but in our country little information is available in this regards. Thus it is important to identify the associated fungi with the diseased rose plant in Bangladesh. In Bangladesh, the most common diseases of this plant are Botrytis blight (*Botrytis cinera*), Cercospora leaf spot (*Cercospora puderi*), Rose mosaic (Rose mosaic virus), Black spot (*Diplocarpon rosae*), Die-back (*Botryodiplodia theobromae*), Alternaria leaf spot (*Alternaria alternata*) and stem canker (*Crytosporrella umbrina*) (Islam *et al.* 2010). In the world, at least 48 fungal species are reported that are capable of causing rose diseases (Wikipedia 2013). Considering importance of the disease, the present investigation was conducted to identify fungal pathogens associated with the diseased rose plant under Bangladesh conditions.

MATERIALS AND METHODS

The present study is based on healthy and infected plant parts of *Rosa* spp. Plant materials were collected from Dhanmondi, Gulshan, Farmgate, Mirpur, Mohakhali and Dhaka University Campus, during November 2009 to October 2010 (Anita 2010). Thirty nine samples were studied in search of fungal diseases of rose plant. In this investigation five types of symptom were recorded on two varieties of rose plant *viz.* *Rosa centifolia* (red, pink and white flower) and *R. involucrata*. The symptoms were Black spot, Leaf spot₁ (spot reddish brown, sub circular 2 - 3 mm in diam.), Leaf spot₂ (off white centre surrounded by reddish brown border 3 - 5 mm in diam. Sub circular), blight and anthracnose.

The fungi were isolated from the samples following the "Tissue Planting method". The selected area of specimens were cut into small pieces (2 mm × 2 mm) and surface sterilized by dipping in 10% chlorox for 3 - 5 min followed by rinsing in sterilized water. Surface sterilized plant pieces were placed on PDA medium. From each sample 30 inocula consisted of leaf pieces were taken and placed on solidified PDA in Petri dishes at 3 pieces per plate. The plates were incubated for 5 - 7 days at 25 ± 1°C. Fungi grew from the inocula were transferred to separate PDA plates and PDA slants for further studies and preservation. The isolated fungi were identified based on morphological characteristics observed under a compound microscope following standard keys (Barnett and Hunter 2000, Booth 1971, Ellis 1971, 1976, Ellis and Ellis 1997, Sutton 1980). Prevalence (%) of fungi in different specimens was also recorded.

All diseased specimens and associated fungi were preserved in the Herbarium, Mycology and Plant Pathology Division, Department of Botany University of Dhaka.

Identified fungi were purified and their pathogenicity was examined following modified 'detached leaf technique' (Azad and Shamsi 2011). Healthy and mature leaves of *Rosa* spp. were collected and washed with distilled water then surface sterilized with 10% Clorox for five minutes and rinsed in sterilized water. Ventral and dorsal sides of the leaflets with and without pricking were inoculated with spores of the isolated fungi. Another set of leaves with and without pricking and without inoculation were maintained, which served as controls. Three replications were made for each treatment. The inoculated leaflets were placed in Petri dishes containing water soaked cotton bar to maintain sufficient humidity to initiate infection. The plates were incubated at 25 - 28°C. After 3 days of inoculation, examination of leaves for pathogenicity test was started and continued for 7 - 10 days for disease development.

Healthy seedling of rose plant was transplanted in pots (30 cm diam.) containing sterilized soil at three seedlings per pot and allowed to grow for three months in net house providing necessary water and nutrients. Healthy leaves of the seedlings were washed with sterilized distilled water and then surface sterilized with 10% chlorox and again washed with sterilized distilled water. Pricked and unpricked leaves were inoculated. Surface sterilized leaves were pricked with sterilized needle. For inoculation 5 mm (diam.) mycelial block cut from young PDA culture of each test fungus and rubbed on both pricked and unpricked leaves and wrapped with surface sterilized polythene bags. Leaves under control received only fresh PDA block without fungal inoculum. Three leaves were inoculated for each treatment and for each fungus. The inoculated plants were placed on a clean bench following completely randomized design.

The plants were examined daily and continued for 10 days to record the development of symptoms. Symptom produced on artificial inoculated leaves were recorded and compared with those observed on naturally inoculated leaves. The fungi were re-isolated from the inoculated leaves of rose plant on PDA medium to fulfill Koch's postulates.

RESULTS AND DISCUSSION

During November, 2009 to October, 2010 five types of symptoms were recorded on two varieties of rose plant viz. *Rosa centifolia* (red, pink and white flower) and *Rosa involucrata*. The symptoms were black spot, leaf spot₁ (spot reddish brown, sub circular 2 - 3 mm in diam.) Leaf spot₂ (off white centre surrounded by reddish brown border 3 - 5 mm in diam. sub circular), blight and anthracnose (Figs 1 and 2).

Present study revealed the presence of association of 21 species of fungi belonging to 17 genera. The isolated fungi were *Alternaria alternata* (Fr.) Keissler, *Arthrinium saccharicola* Stevenson, *Aspergillus flavus*, Link., *A. niger* van Tiegh., *Botrytis allii* Munn, *Cercospora* sp., *Cladosporium cladosporioides* (Fresen.) de Vries, *C. oxysporum*

Berk. & Curt., *Colletotrichum* spp., *Curvularia brakyospora* Boedijn, *Curvularia pallescens* Boedijn, *Fusarium* sp., *Epicoccum purpurascens* Ehreneb ex Schlecht; Link, *Gibberella* sp. and *Marssonina rosea* (Lib.) Died, *Nigrospora sphaerica* (Sacc.) Masson, *Pestalotiopsis guepinii* (Desm.) Stay. with its two culture types, *Penicillium* sp., *Rhizopus stolonifer* (Ehrenb. Ex. Fr) Vuill. and *Trichoderma viride* Pers. ex Fries.

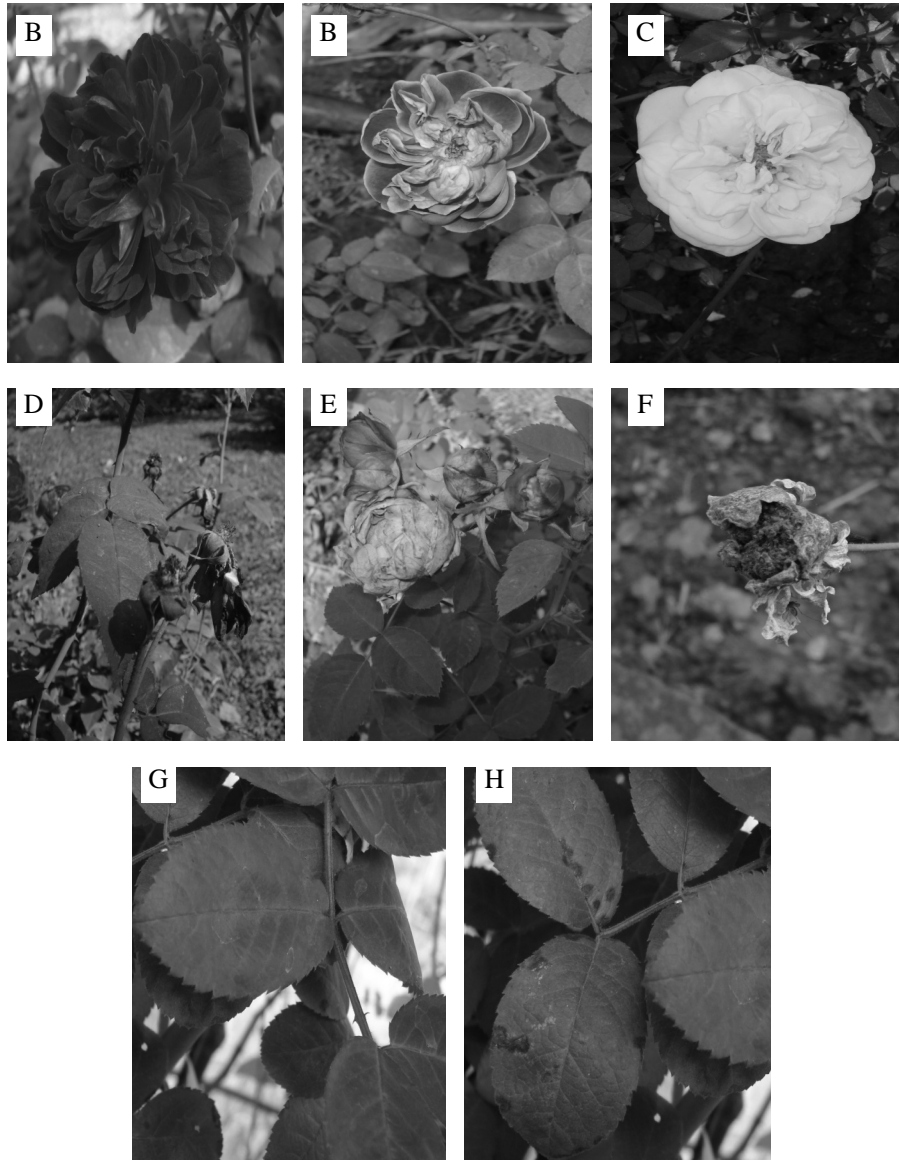


Fig. 1. Healthy and infected plant of *R. centifolia* : A-B. red; C-D. pink and E-F. white flower and G-H. *R. involucrata*.

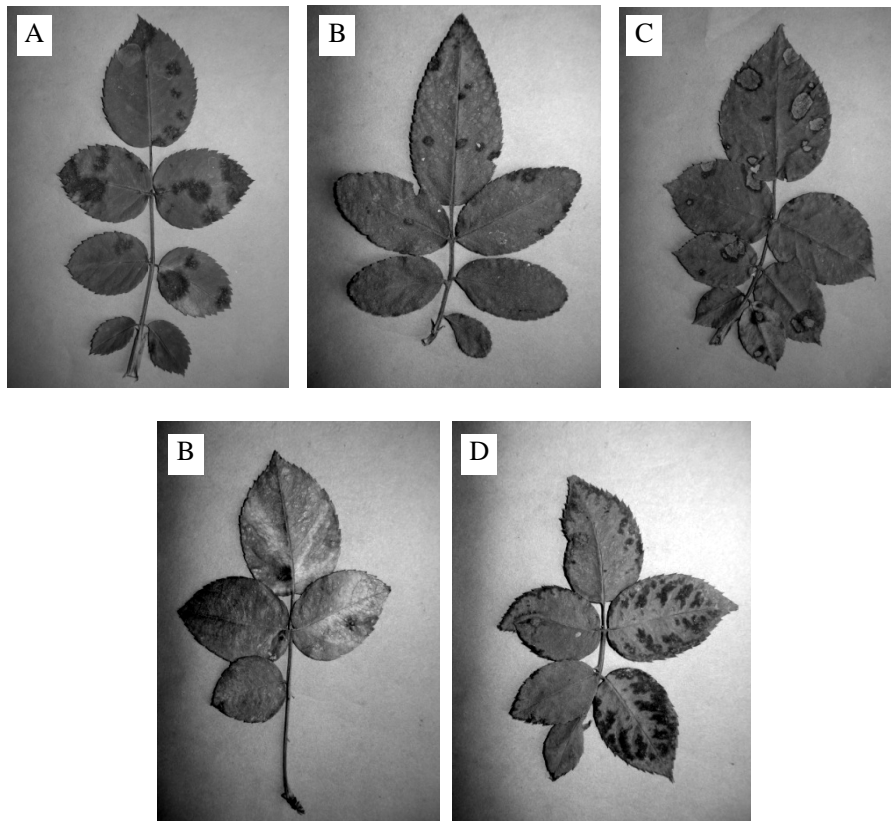


Fig. 2. Symptoms on *Rosa* spp.: A. Black spot. B. Leaf spot₁, C. Leaf spot₂. D. Blight and E. Anthracnose.

In case of healthy leaves of *R. centifolia* (red flower) 5 fungal species were isolated. The fungi were *A. niger*, *C. cladosporioides*, *C. oxysporum*, *Penicillium* sp. and *T. viride*. The frequency percentage of association of *C. oxysporum* was higher (18.7) and lower (5.33) in *A. niger*.

Five fungal species were associated with black spot symptom of *R. centifolia* (red flower). The associated fungi were *C. cladosporioides*, *C. oxysporum*, *Marsonina rosea*, *Penicillium* sp. and *P. guepinii*. The frequency percentage of association of *M. rosea* was higher (30.0), followed by *P. guepinii* (25.33) and lower (4.0) in *Penicillium* sp.

Six fungal species were associated with blight symptom of *R. centifolia* (red flower). The fungi were *A. alternata*, *A. flavus*, *C. cladosporioides*, *Penicillium* sp., *P. guepinii* and *T. viride*. The frequency percentage of association of *Penicillium* sp. was higher (18.33) and lower (3.33) in *T. viride*.

Nine fungal species were associated with anthracnose symptom of *R. centifolia* (red flower). The fungi were *A. alternata*, *A. saccharicola*, *B. allii*, *C. cladosporioides*, *C. oxysporum*, *Colletotrichum* sp.₁ (straight spored), *N. sphaerica*, *P. guepinii*, *P. guepinii*₁ (culture type 1) and *Rhizopus stolonifer*. The frequency percentage of association of *C. cladosporioides* was higher (30.0) and lower (3.33) in *P. guepinii* and *P. guepinii*₁.

Eight fungal species were associated with leaf spot₂ of *R. centifolia* (red flower). The fungi were *A. niger*, *C. cladosporioides*, *C. oxysporum*, *C. pallescens*, *Gibberella* sp., *Penicillium* sp., *P. guepinii*, *P. guepinii*₁ (culture type 1), *P. guepinii*₂ (culture type 2) and *T. viride*. The frequency percentage of association of *P. guepinii*₁ was higher 24.0, and lower 2.66 in *Penicillium* sp. (Table 1).

Table 1. Prevalence of different fungi associated with healthy and infected leaves of *R. centifolia* (red flower).

Fungal isolates	<i>Rosa centifolia</i> (red flower)				
	Healthy	Black spot	Blight	Anthracnose	Leaf spot ₂
<i>Alternaria alternata</i>	-	-	8.33	3.33	-
<i>Arthrinium saccharicola</i>	-	-	-	6.70	-
<i>Aspergillus flavus</i>	-	-	1.66	-	-
<i>A. niger</i>	5.33	-	-	-	1.33
<i>Botrytis allii</i>	-	-	-	3.33	-
<i>Cladosporium cladosporioides</i>	10.50	22.66	16.66	23.33	17.33
<i>Colletotrichum</i> sp. ₁ (straight spored)	-	-	-	20.00	-
<i>Cladosporium oxysporum</i>	18.70	6.66	-	3.00	1.33
<i>Curvularia pallescens</i>	-	-	-	-	1.33
<i>Fusarium</i> sp.	-	-	-	-	-
<i>Marssonina</i> sp.	-	30.00	-	-	-
<i>Nigrospora sphaerica</i>	-	-	-	-	-
<i>Penicillium</i> sp.	13.33	4.00	18.33	-	2.66
<i>Pestalotia guepinii</i>	-	25.33	6.66	3.33	17.33
<i>Pestalotia guepinii</i> ₁	-	8.00	5.00	3.33	24.00
<i>Pestalotia guepinii</i> ₂	-	4.00	5.00	-	6.66
<i>Rhizopus stolonifer</i>	-	-	-	10.00	-
<i>Trichoderma viride</i>	6.70	-	3.33	-	9.33

In case of healthy leaves of *R. centifolia* (white flower) 4 fungal species were isolated. The fungi were *A. saccharicola*, *C. cladosporioides*, *C. oxysporum*, *Penicillium* sp. and *N. sphaerica*. The frequency percentage of association of *Cladosporium* spp. was higher 20.00 and lower 11.5 in *A. saccharicola*.

Four fungal species were associated with leaf spot₁ of *R. centifolia* (white flower). The associated fungi were *A. saccharicola*, *Fusarium* sp., *P. guepinii*, *P. guepinii*₁ (culture type 1) and *T. viride*. The frequency percentage of association of *P. guepinii* was higher (46.66) and lower 3.33 in *Fusarium* sp. and *T. viride*.

In case of leaf spot₂ of *R. centifolia* (white flower) four fungal species were associated. The fungi were *C. cladosporioides*, *E. purpurascens*, *N. sphaerica*, *P.*

guepinii and *P. guepinii*₁ (culture type 1). The frequency percentage of association of *N. sphaerica* was higher 46.66, and lower 6.66 in *C. cladosporioides* (Table 2).

Table 2. Prevalence of different fungi associated with healthy and infected leaves of *R. centifolia* (white flower).

Fungal isolates	Healthy	Leaf spot ₁	Leaf spot ₂
<i>Arthrinium saccharicola</i>	11.5	20.00	-
<i>Cladosporium cladosporioides</i>	20.00	-	6.66
<i>Cladosporium oxysporum</i>	20.00	-	-
<i>Epicoccum purpurascens</i>	-	-	9.10
<i>Fusarium</i> sp.	-	3.33	-
<i>Nigrospora sphaerica</i>	19.23	-	46.66
<i>Pestalotia guepinii</i>	-	46.66	20.00
<i>Pestalotia guepinii</i> ₁	-	23.33	10.00
<i>Trichoderma viride</i>	-	3.33	-

Five fungal species were associated with healthy leaves of *R. centifolia* (pink flower). The fungi were *A. alternata*, *A. flavus*, *C. cladosporioides*, *C. oxysporum* and *P. guepinii*₁ (culture type 1). The frequency percentage of *P. guepinii*₁ (culture type 1) was higher 30.00 and lower 7.4 in *A. alternata*,

Table 3. Prevalence of different fungi associated with healthy and infected leaves of *R. centifolia* (pink flower) and *R. involucrata*.

Fungal isolates	<i>Rosa centifolia</i> (pink flower)			<i>R. involucrata</i>	
	Healthy	Black spot	Infected stem	Healthy	Leaf spot ₁
<i>Alternaria alternata</i>	7.40	4.76	-	3.33	6.70
<i>Arthrinium saccharicola</i>	-	-	-	-	-
<i>Aspergillus flavus</i>	10.00	2.38	7.14	-	-
<i>Cladosporium cladosporioides</i>	14.81	40.47	-	6.70	20.00
<i>C. oxysporum</i>	11.11	2.56	-	3.33	13.33
<i>Cercospora</i> sp.	-	-	-	-	13.33
<i>Colletotrichum</i> sp. ₂ (curved spored)	-	-	28.00	-	-
<i>Curvularia brachyospora</i>	-	28.20	-	-	-
<i>C. pallenscens</i>	-	2.38	-	-	-
<i>Marssonina rosea</i>	-	20.00	-	-	-
<i>Nigrospora sphaerica</i>	-	-	26.78	13.33	13.33
<i>Penicillium</i> sp.	-	2.38	7.14	13.33	20.00
<i>Pestalotia guepinii</i>	-	16.66	39.28	-	23.33
<i>P. guepinii</i> ₁	30.00	16.66	80.35	-	13.33
<i>P. guepinii</i> ₂	-	-	13.39	-	16.70

'-' = No fungal growth.

Nine fungal species were associated with black spot symptom *R. centifolia* (pink flower). The fungi were *A. alternata*, *A. flavus*, *C. cladosporioides*, *C. oxysporum*, *C.*

brachyspora, *C. pallescens*, *M. rosea*, *Penicillium* sp., *P. guepinii* and *P. guepinii*₁ (culture type 1). The frequency percentage of association of *C. cladosporioides*₁ was higher 40.47 and lower 2.38 in *A. flavus* and *Penicillium* sp.

Five fungal species were associated with stem of *R. centifolia* (pink flower). The fungi were *A. flavus*, *Colletotrichum* sp.₂ (curved spored), *N. sphaerica*, *Penicillium* sp., *P. guepinii*, *P. guepinii*₁ (culture type 1) and *P. guepinii*₂ (culture type 2). The frequency percentage of association of *P. guepinii*₁ (culture type 1) was higher (80.35) and lower (7.14) in *A. flavus* and *Penicillium* sp. (Table 3).

Five fungal species were associated with healthy leaves of *R. involucreta*. The fungi were *A. alternata*, *C. cladosporioides*, *C. oxysporum*, *N. sphaerica* and *Penicillium* sp. The frequency percentage of *N. sphaerica* and *Penicillium* sp. was higher (13.33) and lower (3.33) in *A. alternata*,

Seven fungal species were associated leaf sp₁ symptom *R. involucreta*. The fungi were *A. alternata*, *C. cladosporioides*, *C. oxysporum*, *Cercospora* sp., *N. sphaerica*, *Penicillium* sp., *P. guepinii*, *P. guepinii*₁ (culture type 1) and *P. guepinii*₂ (culture type 2). The frequency percentage of association of *P. guepinii* was higher (23.33) and lower (6.7) in *A. alternata*.

Diplocarpon rosae Wolf with its imperfect stage *Marssonina rosae* (Lib.) died is the causal agents of black spot disease (William 1949, Debner *et al.* 1988, Wikipedia 2013). The spots are circular with a perforated edge and reach a diameter of about 14 mm, badly affected plants however, will not show the circular spots as they combine to cause black mass.

Diplocarpon rosae and its imperfect stage *Marssonina rosae* are well documented pathogens of black spot of rose but in the present study in addition to the above mentioned two fungi *Pestalotiopsis guepini* and its two culture types were frequently isolated from infected leaves and stem of *R. centifolia* and *R. involucreta*. *Pestalotiopsis guepinii*₁ was also isolated from healthy leaves of *R. centifolia* (pink flower).

Pestalotiopsis guepinii has wide host range. From Bangladesh the fungus was previously isolated from *Aloe vera* L. and *Sinna atata* L. (Shutrothor and Shamsi 2013 and Shamsi. *et al.* 2013).

From Bangladesh this is the first report of association of *A. saccharicola*, *A. flavus*, *A. niger.*, *B. allii*, *C. cladosporioides*, *C. oxysporum.*, *Colletotrichum* sp., *Curvularia brakyospora*, *C. pallescens*, *Fusarium* sp., *E. purpurascens*, *Gibberella* sp. *N. sphaerica*, *Pestalotiopsis guepinii* (Desm.) Stay. with its two culture types, *Penicillium* sp., *R. stolonifer* and *T. viride* with rose plant.

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