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SEASON INCIDENCE AND DISTRIBUTION OF VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI COLONIZATION IN ROOTS AND ITS ASSOCIATED RHIZOSPHERE AND PHYLLOSPHERE MYCOFLORA OF A MEDICINAL PLANT *Coleus forskohlii*

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ABSTRACT

A survey was conducted in Department of Botany, Government General Degree College, Singur during the year 2019-2020 to assess the seasonal occurrence and distribution of vesicular arbuscular mycorrhizal (VAM) fungi including roots and rhizosphere and phyllosphere mycoflora of a medicinal plant *Coleus forskohlii* (Wild.) Briq. Mycorrhizal spore count increased continuously from June which reached a maximum in August (monsoon season). It decreased steadily from November to February (winter) and moderate in summer (March to May). Spore count was minimum in January and February (winter). Similarly root colonization by VAM fungi was maximum during monsoon, moderate during summer and low during winter.*Glomus* spp. were dominant VAM fungi over others at the study site. Rainy season was best for effective colonization and establishment of VAM fungi in the experimental field. Population studies of the rhizosphere and phyllosphere mycoflora, *Fusarium oxysporum*, the wilt pathogen of *Coleus*, occurred repeatedly only in the rhizosphere soil in all cases under study. From the population studies, *Aspergillus, Alternaria, Penicillium* and *Curvularia* species were found to be present in both regions where *Aspergillus niger* was the main competitor.

(Key words: Seasons, VAM colonization, VAM spore, *Coleus forskohlii,* Rhizosphere, Phyllosphere mycoflora)

INTRODUCTION

Vesicular arbuscular mycorrhizal fungi form symbiotic associations with most economically important plants (Chen *et al.,* 2018). These fungi improve plant growth under low fertility conditions, confer tolerance to some plant pathogens, improve plant water balance, contribute to soil structure and help plants establish in new areas (Panwar and Vyas, 2002). VAM colonization varies with seasonal changes. Seasonal effects also affect plant establishment under field conditions, depending on the performance of native VAM fungi (Basiru and Hijri, 2022). Information on seasonal variation in spore count and root colonization of VAM fungi is useful for timely inoculation of appropriate species.VAM fungi are known to improve plant nutritional status, growth, development and protect plants against root pathogens (Vyas and Shukla, 2005) and offer drought resistance (Auge and Moore, 2005). Although these fungi are not host specific, recent studies have clearly revealed host dominance of AM fungi thus emphasizing the need to select efficient AM fungi to inoculate a specific host. Some medicinal plant species like *Phyllanthus amarus* and *Withania somnifera* (Earanna, 2001), *Coleus foskohlii* (Gracy and Bagyaraj, 2005), *Andrographis paniculata* and medicinal plants of Apocynaceae and Asclepiadaceae (Swarupa Rani and Manoharachari, 2007) host preferences are reported. These fungi produce unique structures called vesicles, arbuscules and hyphae inside the root cortices while hyphae spores/sporocarps and sometimes external vesicles are present outside the root. *Coleus forskohlii* is the most promising medicinal crop of the future, because of its recently discovered pharmacoproperties. Its tuberous roots are found to be a rich source of forskolin. An increase in forskolin (active ingredient) content of *C. forskohlii* after AM inoculation was recently reported (Boby and Bagyaraj, 2003). Effect of different AM-fungi on growth, nutrition and forskolin content of *C. forskohlii* was also reported by Gracy and Bagyaraj (2005), Borkotoki *et al.* (2019) and Khatun (2020a). Biological control of *C. forskohlii* root rot using microbial inoculants was observed by Bobby and Bagyaraj (2003), Bandopadhyay *et al.* (2019) and Khatun (2020b). VAM colonization varies with seasonal changes (Sharma *et al.,* 2013). Seasonal effects also affect plant establishment under field conditions, depending on the efficiency of native VAM fungi (Lugo and Cabello, 2002; Sharma *et al.,* 2005).Soil-borne plant pathogens pose a major problem for strategic management of diseases. Soil-borne diseases are

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difficult to control and seed treatment with fungicides does not protect crops for long. Continuous use of the same fungicide leads to the development of fungicide resistant strains of pathogens and application of fungicides to soil is costly and harmful to the associated soil microbiota (Bunker and Mathur, 2001). Bio-control agents are gaining ground in plant pathogen management as an alternative to chemical fungicides in recent times (Manoranjitham *et al.,* 2002). Kesherwani *et al.* (2018) reported that seed borne mycoflora i.e. *Rhizopus* sp. and *Fusarium* sp. reduced the seedling vigour index of pea varieties whereas, fungicidal and biocontrol agent *Trichoderma* sp. increased the seedling vigour index by keeping seed borne mycoflora under check. Biological management of soil-borne diseases is gaining increasing status as a potentially practical and safe method (Patel and Anahosur, 2001). The paper deals with the seasonal variation in the presence of VAM fungi and the intensity of mycorrhization in roots and its rhizosphere and phyllosphere mycoflora of the medicinal plant *C.forskohlii*.

MATERIALS AND METHODS

Study site

The study on *Coleus forskohlii* was conducted in the departmental garden of Government General Degree College, Singur during the year 2019-2020. The climate of the area is tropical. Winter starts from mid-November and continues till the end of February. The dry summer from March to May is interrupted by tropical cyclones and storms. June to September is the rainy season, warm and humid, and October and November are autumn. The average temperature in hot season is 30^0 C and in cold season it is 20^0 C. The average rainfall in the area is 150 mm. The soil texture is sandy with a pH of 7.0 (neutral), total phosphorus 55–65 ppm, total nitrogen 0.02 to 0.03%, and organic carbon 1.1–1.05%.

Sampling

Fine roots and rhizospheric soil of *C. forskohlii* were collected from experimental gardens of the department at the end of every month. Five individual plants of *C. forskohlii* of approximately the same age were randomly sampled from the site. Fine roots (1.5-2.0 mm diam.) and rhizospheric soil samples (15-30 cm deep) were collected from each plant and stored in polythene bags at 5^0C for further analysis. Five replicates were made for each sample.

Estimation of VAM spore population

VAM spores were isolated from soil samples using the 'wet sieving and decanting technique' (Gerdermann and Nicolson, 1963) and quantified using the 'grid-line intersect method' (Adholeya and Gaur, 1994).

Estimation of VAM root colonization

Root colonization of *C.forskohlii* by mycorrhizal fungi was observed by 'Rapid clearing and staining technique' (Philips and Hayman, 1970). Per cent root colonization (Figure 1) was quantified based on the number of root segments colonized by VAM fungi as follows: Percentage mycorrhization of root

$$\text{colonization}=\left[\frac{\text{Total no. of roots colonized}}{\text{Total no. of roots studied}}\times 100\right]$$

Identification of VAM fungal spores

Intact spores were examined and identified using the manual of Schenck and Perez (1987) and Morton and Benny (1990). The data regarding rate of VAM fungal colonization in roots and spore population in the rhizosphere of *C. forskohlii* are presented in Figure 2.

Collection of soil samples with subsequent isolation and identification of rhizosphere mycoflora

Rhizosphere soil samples (10-15 g) were collected in fresh polythene bags (15 × 10 cm) from both the infected and healthy *Coleus* fields of Hooghly district. Soil dilution and plate count method of Aneja (1993) were adopted for isolation of mycoflora from rhizosphere soil samples. One gram of air-dried soil sample was weighed and suspended in 100 ml of sterile distilled water and was shaken uniformly with the help of a magnetic shaker for 15 minutes. 0.5 ml of soil suspension at 10^{-6} dilution was poured on 15 ml solidified potato dextrose agar (PDA) medium in petridishes. Five replica plates were maintained in each case. The petriplates were incubated at $28^0\pm1^0$C for 2 to 4 days under observation. The fungal colonies were isolated and pure lines of the isolates were made. The isolates were identified (Table 1) [Nagamani *et al.,* 2006, Gilman and Joseph, 1988] and maintained on PDA slants at 4^0C for future uses.

Isolation and identification of phyllosphere mycoflora

In order to isolate phyllosphere mycoflora, fresh and healthy *Coleus* leaves were collected randomly from different fields of Hooghly district under cultivation and kept them in fresh sterilized polypropylene bags. By using a sterile cork borer, the leaves were cut into discs (5 mm) and were transferred to 100 ml of sterilized distilled water with the help of a sterile forcep and stirred for 25 minutes. After removal of the leaf discs, the solution was diluted upto 10^{-2} dilutions. 0.5 ml of each of these dilutions was poured on 15 ml solidified potato dextrose agar (PDA) medium in petridishes. Five replica plates were maintained in each case. The petriplates were incubated at $28^0\pm1^0$C for 2 to 4 days under observation. The fungal colonies were isolated and pure lines of the isolates were made. The isolates were identified (Nagamani *et al.,* 2006, Gilman and Joseph, 1988) and maintained on PDA slants at 4^0C for future uses. The phyllosphere mycoflora are enlisted in Table 2.

RESULTS AND DISCUSSION

The rate of VAM fungal colonization in roots and spore population in the rhizosphere of *C. forskohlii* showed wide range of variation in different seasons (Figure 2). In *C. forskohlii*, mycorrhizal spore counts steadily increased from

June reaching maximum (35.2 spores 50 g^{-1} of soil) in August (rainy season). It defined steady from November to February (winter) and moderate in summer (March to May). Spores counts remained minimum in January and February (4.8 spores 50 g^{-1} of soil and 5.6 spores 50 g^{-1} of soil respectively) in winter. However, it is apparent from the result that the maximum abundance of VA-mycorrhizal spores was recorded during rainy season. VAM spores isolated from the rhizosphere soil of *C. forskohlii* were identified as *Glomus fasciculatum, Glomus mosseae, Glomus* spp. and *Gigaspora* sp. Colonization of VAM fungi in roots of *C. forskohlii* remained considerably high (Figure 2) during the rainy season being maximum in the month of August (100%).

Collection of samples, and subsequent isolation and identification of rhizosphere and phyllosphere mycoflora

Through population studies of rhizosphere and phyllosphere mycoflora (Table 1 and 2), *Fusarium oxysporum*, wilt pathogen of *Coleus* was found to perennate only in the rhizosphere soil in all the fields under study. From population studies, species of *Aspergillus*, *Alternaria*, *Penicillium* and *Curvularia* were found to be present both in the regions where *Aspergillus niger* remained the predominant competitor.

Seasonal variation of VAM colonization in roots and rhizosphere soil of the plant

The rate of VAM fungal colonization in roots and spore population in the rhizosphere of *C. forskohlii* showed wide range of variation in different seasons [Figure 2]. In *C. forskohlii*, mycorrhizal spore counts steadily increased from June reaching maximum (35.2 spores 50 g^{-1} of soil) in August (rainy season). It defined steady from November to February (winter) and moderate in summer (March to May). Spores counts remained minimum in January and February (4.8 spores 50 g^{-1} of soil and 5.6 spores 50 g^{-1} of soil respectively) in winter. However, it is apparent from the results that the maximum abundance of VA-mycorrhizal spores was recorded during rainy season. VAM spores isolated from the rhizosphere soil of *C. forskohlii* were identified as *Glomus fasciculatum, Glomus mosseae, Glomus* sp. and *Gigaspora* sp. Colonization of VAM fungi in roots of *C. forskohlii* remained considerably high (Figure 2) during the rainy season being maximum in the month of August (100%). *Glomus* was found to be predominant genus associated with rhizospheric soil of *C. forskohlii* at neutral pH 7.0. *Glomus* sp. extracted from a neutral soil showing acidic and alkaline pH has been reported by Selvaraj *et al.* (2001).A wide range of variation in root colonization might be due to the effect of rhizosphere soil of *C. forskohlii* that favoured growth of VAM fungi differently in different season (Sharma *et al.,* 2005). The levels of VAM fungi association depend on root morphology, metabolism rate of plant growth (Mangla *et al.,* 2022) as well as on specific soil plant system, chemical nature of root exudates (Wahab *et al.,* 2023). In addition to these factors, pH of the soil may play a role in regulating the VAM root colonization and spore count. In natural system Lugo and Cabello (2002), Bohrer and Amon (2004), Sharma *et al.* (2005) observed that seasonal fluctuations of mycorrhizal associations were closely related to plant phenology which corroborates the present findings. In *C. forskohlii* maximum spore count was observed in August and September (35.2 spores 50 g^{-1} of soil and 21.6 spores 50 g^{-1} of soil) which coincides with the flowering time of *C. forskohlii.* VAM sporulation and plant flowering might be related because at this time, host photosynthate is allocated to roots and rhizomes, which helps the fungal symbiont to produce more spores (Sharma *et al.,* 2005). The VAM associations were more in rainy season because roots showed better growth during this season and active root growth provided more entry points to VAM fungi (Sharma *et al.,* 2005). Spore count drastically gets declined in winter which coincided with the senescence of the host when root growth was very slow (Sharma *et al*., 2005). Similar result was also reported by Sharma *et al.* (2005), who observed that the fruiting stage reduces carbohydrate availability to the fungus, which results in decreased production of spores. Similar observation was recorded in *C. forskohlii* during winter (November to January), which coincides with fruiting stage of this plant. On the other hand, foliage senescence in winter resulted in reduced carbohydrate translocation of roots which led to minimum colonization in that season. Sharma *et al.* (2005) was observed that VAM colonization was less in winter remained moderate in summer and increased in the rainy season. Similarly, the most VAM spores were found in rainy season, moderate numbers in summer and the least in winter. Boby *et al.* (2008) was found that mycorrhizal root colonization, spore numbers and population of yeasts in the root zone soil were also highest in the treatment *Glomus mosseae*+ *Rhodotorula mucilaginosa* and least in the uninoculated plants.

Isolation and identification of rhizosphere and phyllosphere mycoflora

It is evident from the result (Table 1 and 2) that the pathogen, *F. oxysporum* is a soil inhabitant. The pathogen invades the plant mainly through the roots and between the crops it survives in infected plant debris in the soil as mycelium and in all its spore forms but most commonly as chlamydospores (Raaijmakers *et al.,* 2009).

It is apparent that out of the total sixteen rhizosphere fungi and ten phyllosphere fungi, species of *Aspergillus* and *Penicillium* were most frequent in their occurrence. Previous authors (Kanjilal, 2006; Ojha, 2008) reported that *Aspergillus* spp. and *Penicillium* spp. were the common coloniser of rhizosphere soils in tomato plants. Three species of *Trichoderma* were isolated from rhizosphere and these species were found to be antagonistic against the pathogen through subsequent trial experiments. Beneficial microorganisms present in the rhizosphere restrict the growth of soil borne pathogens (Shah *et al.,* 2005). Microorganisms that can grow in the rhizosphere are ideal for use as biocontrol agents, since the rhizosphere provides the front-line defense for roots against attack by pathogens. Pathogens encounter antagonism from rhizosphere microorganisms before and during primary infection and also during secondary spread on the root (Ojha, 2008). The region surrounding the mycorrhizal root is called the mycrorhizosphere, the mycorrhizosphere is based on the fact that mycorrhizae have a strong influence to the microflora of the rhizosphere (Johansson *et al.,* 2004), resulting in the association of various types of bacteria and nematodes in rhizosphere VAM and non VAM plants and alters the mechanism of action of which is not yet known. The present study reveals that seasonal variation of VAM fungi showed tripartite correlation between the VAM root colonization, spore production and host plant growth. The rainy season (July to September) is best for efficient activity of VAM fungi at field levels in *C. forskohlii.* From population studies, species of *Aspergillus, Alternaria, Penicillium* and *Curvularia* were found to be present both in the regions where *Aspergillus niger* remained the predominant competitor.

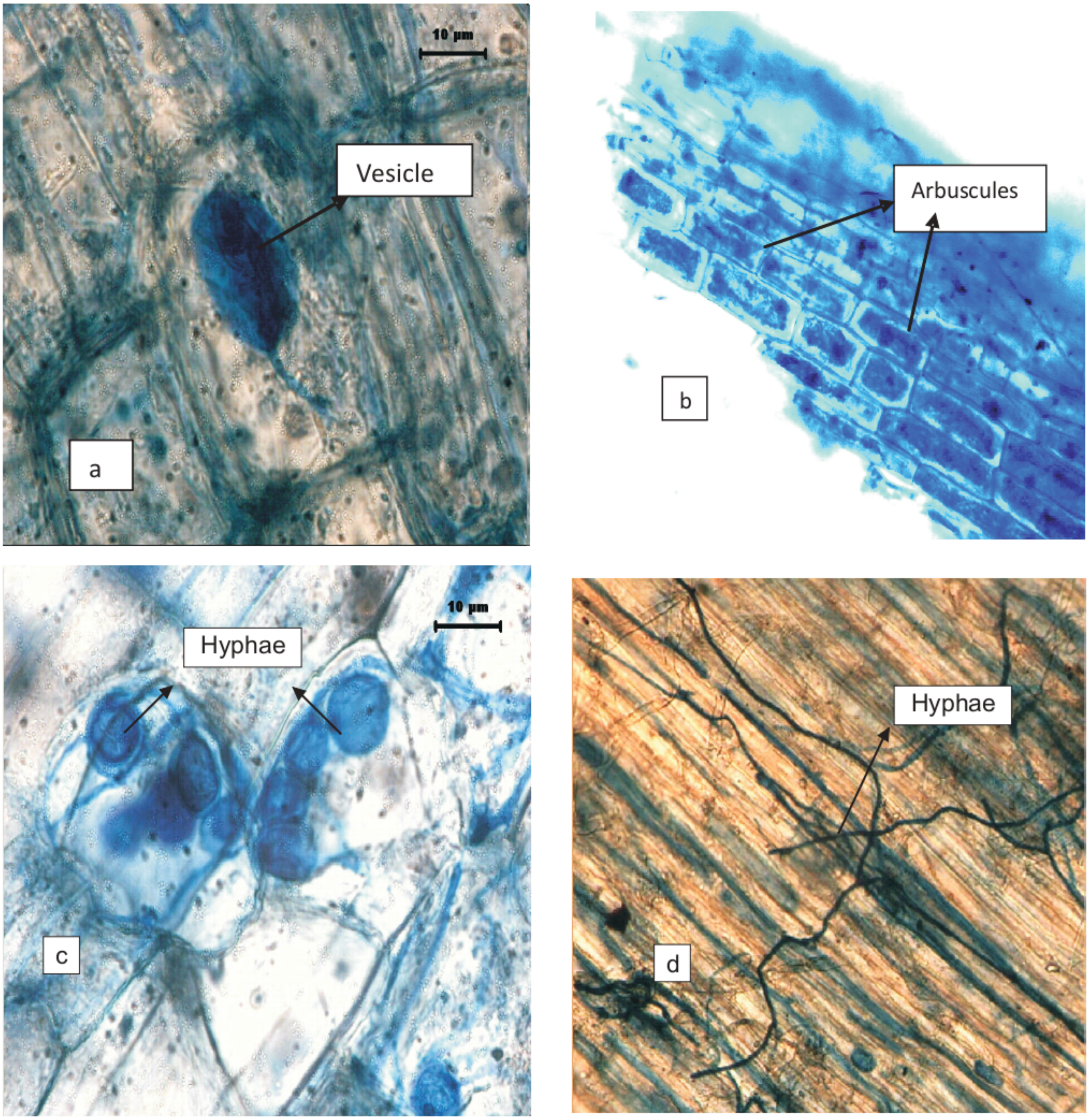

Figure 1. Photographs showing mycorrhizal root colonization of the *Coleus* plant (a) vesicle present within the cortex cell of the root, (b) arbuscules present within the cortex cells of the root Bar= 10µm, (c) spores present within the cortex cells of the root. and(d) mycorrizal hyphae present within the cortex cells of the root Bar= 10µm

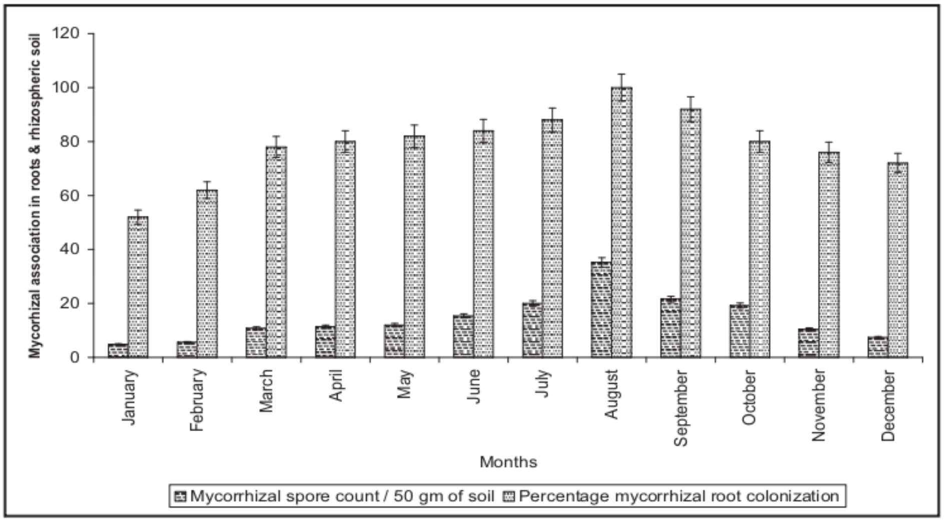

Figure 2. Seasonal variation of mycorrhizal association in *C. forskohlii.* Each value is the mean± SE for n = 5.Bars represent standard error

Table 1. Fungal isolates from rhizosphere of *Coleus* plant ('+'/'-'indicates presence/absence)

Isolates	Fungi	Field No. 1	Field No. 2	Field No. 3	Field No. 4	Field No. 5
1	*Penicillium* sp.	+	+	-	-	+
2	*Trichoderma reesei*	-	+	-	+	+
3	*Fusarium solani*	+	-	-	+	+
4	*Trichoderma viride*	+	+	+	+	+
5	*Fusarium oxysporum*	+	+	+	+	+
6	*Alternaria* sp.	+	-	-	+	+
7	*Aspergillus niger*	+	+	+	-	-
8	*Penillium citrinum*	-	-	-	-	+
9	*Botryodiplodia theobromae*	+	-	-	-	-
10	*Alternaria solani*	-	+	+	+	+
11	*Sclerotium* sp.	-	-	-	+	-
12	*Rhizoctonia solani*	-	+	+	-	-
13	*Pythium* sp.	-	-	-	-	+
14	*Trichoderma lignorum*	+	-	-	+	+
15	*Curvularia* sp.	-	-	-	-	+
16	*Gliocladium* sp.	-	-	-	+	-

Table 2. Fungal isolates from phyllosphere of *Coleus* plant ('+'/'-'indicates presence/absence)

Isolaates	Fungi	Field No. 1	Field No. 2	Field No. 3	Field No. 4	Field No. 5
1	*Alternaria* sp.	+	+	+	-	-
2	*Curvularia* sp.	-	+	-	-	-
3	*Aspergillus* sp.	-	-	+	+	+
4	*Penicillium* sp.	-	+	-	+	+
5	*Mucor* sp.	+	+	-	-	-
6	*Penicillium citrinum*	-	-	-	+	-
7	*Aspergillus niger*	+	+	-	-	-
8	*Phoma* sp.	-	-	-	+	-
9	*Cladosporium* sp.	-	-	+	-	-
10	*Colletotrichum* sp.	+	-	-	-	-

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