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Isolation and Characterization of *Fusarium* species causing leaf spot and root rot diseases on *Aloe vera*

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ABSTRACT

Aloe vera (*Aloe barbadensis* Miller) is a marvelous medicinal plant well known for its excellent medicinal properties. Leaf spot and root rot diseases were found on *Aloe vera* in various areas of Gwalior, Madhya Pradesh, India, in winters of 2011-2012. The typical disease symptoms were observed on both abaxial and adaxial surface of leaves as well as on root. Leaf spot, necrosis, root rot, yellowing of plants and even death was recorded as diseases symptoms. On the basis of morphological and microscopic characteristics of the fungus, three species of *Fusarium* i.e. *F. fusaroides*, *F. moniliforme* and *F. solani*, were found to be associated with the leaf spot and root rot diseases. Koch's postulate was applied to confirm the causal organisms of the diseases. As per literature till date, this seems to be the first report of leaf spot disease on *A. vera* caused by *F. fusaroides* and *F. moniliforme* while second report of root rot by *F. solani* from India.

Key words: *Aloe vera*, India, leaf spot, root rot, *Fusarium* sp.

INTRODUCTION

Aloe vera (*Aloe barbadensis* Miller) is an important medicinal plant cultivated commercially throughout the world due to its distinctive importance in medicinal and cosmetic industry. Being biological in nature, *A. vera* is attacked by a number of fungal and bacterial pathogens which causes numerous diseases. Besides bacteria, fungal pathogens are more likely to be associated with *A. vera* both in natural and artificial conditions and causes diseases like leaf spot, collar and root rot which affects the plant growth, development and therapeutic potential (Lecomteac et al. 2016). The *Aloe vera*

plant is prone to these diseases due to lack of scientific farming technology and favorable abiotic conditions, which favours the growth of pathogens. Till date there are number of reports where *A. vera* plantation has been reported to suffer with several fungal diseases (Roy & Bilgrami 1975; Roy 1976; Gupta et al. 1984; Harsh et al. 1990; Dubey & Pandey 2007; Majumdar et al. 2007). Due to pathogenic infection, the photosynthetic process get disturbed, which affects the plant physiology and influences the survival rate of the plant (Mandal et al. 2009; Lobato et al. 2009; Zhao et al. 2013). These abnormalities may result in increased

susceptibility to opportunistic pests and pathogens. Therefore, not only cultivation, but these diseases also reduce the quality and yield of leaves which are of commercial importance.

Aloe vera (L.) Burm. f. (Aloeaceae) is a unique medicinal plant of dry climates distributed in Asia, Africa and other warm regions of the world. Medicinally, it is very effective in burns, eczema, herpes lesions, rashes, insect bites, stings, psoriasis, sunburn and wound healing (Rajeswari et al. 2012). It improves immunity and is widely used as a major ingredient of various beauty products (Sharma et al. 2013). Keeping in view the importance of plants in medicinal and cosmetic industry, a survey was conducted during the winters of 2011 and 2012 in various areas of Gwalior, Madhya Pradesh. Severe spotting on leaves and drying of *A. vera* plants was noticed in various nurseries and botanical gardens located areas in Gwalior. Further investigations of disease symptoms revealed leaf spot and root rot diseases associated with the plants. This study was done with the objectives to identify the causal agent of leaf spot and root rot symptoms associated with *A. vera*.

MATERIALS AND METHODS

Sample collection and study of symptoms

Diseased samples of *Aloe vera* leaves and roots were collected randomly from each nursery during the survey of various nurseries in Gwalior, placed into labelled zip lock bags and brought into the laboratory. Each diseased sample was studied firstly with hand lens and then with dissecting microscope to assess the morphological characteristics of the disease.

Isolation and purification of pathogen from diseased leaves

Diseased samples were washed thoroughly with running tap water to remove the surface contaminants and cut into small pieces using sterile scalpel blades. These small pieces were then surface sterilized with 2% sodium hypochlorite solution (NaOCl) for 2 mins and then washed three-four times in sterile distilled water. These surface sterilized pieces were then placed between blotting papers and aseptically inoculated onto petridishes containing Potato Dextrose Agar (PDA) media. The plates were incubated at 25±2 °C for 5 to 6 days. The growth of fungal colonies was recorded regularly every day.

Characterization and identification of the pathogens

The isolated fungal species were identified on the basis of morphological and cultural characteristics (shape, size colour and texture of colony) as well as

microscopic features (characteristic of mycelium, shape, size and colour of conidia, etc.) as described by Ellis (1971); Nelson et al. (1983, 1994); Leslie & Summerell (2006); Gilman & Joseph (2008); Moretti (2009). Identification of pathogens was further confirmed at the Indian Type Culture Collection (ITCC), IARI, New Delhi and the National Fungal Culture Collection of India (NFCCI), Agharkar Research Institute, Pune, Maharashtra, India.

Pathogenicity test of the pathogens

The isolated fungi were evaluated for their pathogenicity against *A. vera*. The isolated fungal species were cultured on Potato Dextrose Agar (PDA) medium at 25±2°C for 8-10 days in an incubator. For leaf spot pathogens conidial concentration was subsequently adjusted to 1×10⁹ per ml by using hemocytometer to make a spore suspension. Healthy leaves were surface sterilized for 1 min with 2% sodium hypochlorite solution (NaOCl). Artificial pricks approximately 2 mm deep on the abaxial surface of leaves were made by sterilized needle. Spore suspension of the test organisms was delivered through a sprayer and lined with moist blotting paper. Leaves sprayed with sterile distilled water served as control. Leaves were incubated at 25±2 °C for 8-10 days. In case of root rot disease about four month old plants were grown in 25 cm diameter plastic pots containing autoclaved sandy loam soil artificially infested with the test fungal isolate. Soil was infested by adding 200 ml of hyphal or spore suspension (4×10⁷ ml) of the fungal culture and then filled in plastic pots. A set of pots without fungal inoculum served as control. Soil was irrigated every 3–4 days to ensure equal distribution of fungal inoculum. The experiment was performed in triplicates and pathogenicity was evaluated at 25, 35, 45 and 60 days after inoculation

RESULTS

During the survey, it was found that all the nurseries in Gwalior planted with *Aloe vera* were showing symptoms of leaf spot and root rot. It was observed that leaf spot infection was found frequently while drying of plant due to root rot was also observed significantly. The cultural characteristics of the isolated fungi along with microscopic analysis indicate the association of three *Fusarium* species, *Fusarium fusaroides*, *Fusarium moniliforme* and *Fusarium solani*. Further investigations revealed association of *Fusarium* species with *A. vera* as; *F. fusaroidesi* and *F. moniliforme* with leaf spot and *F. solani* with root rot. The disease symptoms and microscopic characteristics of the pathogens are described as follows.

Symptoms of leaf spot and root rot diseases

Symptoms of leaf spot disease caused by *F. fusaroides* began in the form of circular to oval water soaked maroon spots. Gradually spots became embedded, enlarged and reddish brown in colour. At maturity, diseased tissues become necrotic, spots turned into black in colour and 0.5-1.2×0.4-0.8 cm in diameter. Interestingly, disease was noticed in winter season during survey (January to February).

Leaf spot disease caused by *F. moniliforme* appeared as irregular to sometimes circular lesion on the abaxial or adaxial surface of leaf during winter season. In earlier stage of infection, lesions were sunken and light cream in colour. Progressively, these lesions became enlarged, embedded, creamish brown in colour and had reddish brown margin, varying in size from 1.1-4.0×0.8-3.6 cm in diameter. Ultimately, dark brown sporulation observed on the centre of the spots, two or more lesion coalesces to form a large lesion. Gel of the leaf was mushy and in severe condition diseased portion was broken down. It was recorded in field experiments as well as in survey during December to February.

Symptoms of root rot disease of *A. vera* caused by *F. solani* was interestingly appeared in the rainy season in the form of browning and decaying of root tips. After decaying, symptoms spread towards the distal portion of root resulted in total rotting of the root system and get collapsed. The leaves showed decline and yellowing colouration and later the margin of leaf turned inside due to the dryness of mucilaginous gel. It was recorded only in survey during July to August.

Identification of fungal pathogens

Mycelium on PDA of the first fungus first appears white then soon became pinkish peach in colour. Microconidia were fusiform to clavate with rounded apex and pointed base usually one septate measure 13-15×2-3 µm. Microconidia were curved, fusoid with a narrow round to pointed apex, 4-6 septate measured 40-55×2.5-3.5 µm. Chlamydospores develop usually later and terminal in position (Fig. 1). Based on morphological and cultural characteristics the fungus was identified as *Fusarium fusaroides* (Frag. & Cif.) Booth (# NFCCI-3056).

The second fungus on PDA includes the characteristic feature, reddish-purple pigmentation in culture. Mycelium was hyaline. Conidiophores were medium in size. An abundance of oval microconidia borne in chains, measure 7-10×2.5-3.2 µm. Macroconidia were few, very slightly sickle shaped to nearly straight, 3-7 septate, measuring 31-58×2.7-3µm. Sporodochia bright in mass, chlamydospores were not seen (Fig. 2). Based on morphological and cultural characteristics

the fungus was identified as *Fusarium moniliforme* Sheldon (# ITCC-8187.11).

Colonies of third fungus on PDA showed brownish white to loam yellow, creamish-white mycelium. Macroconidia have three to five septa, twisted spindle form, slightly curved and have a slightly blunted apical end measuring 19-50×2.5-3.0 µm. Microconidia were abundant, oval shaped, and formed in false heads on very long monophialides. Chlamydospores were terminal and intercalary, globose to pear shaped (Fig. 3). Based on morphological and cultural characteristics the fungus was identified as *Fusarium solani* (Mart.) Sacc. (# NFCCI-3052).

Pathogenicity Test

Under in-vitro conditions *Fusarium fusaroides* and *F. moniliforme* were pathogenic. The symptoms of leaf spot diseases recorded during the pathogenicity test were almost similar to the natural symptoms. Symptoms of leaf spot infection appeared on fourth day of infestation. Initially, small circular to irregular, water soaked spots were appeared on the leaf surface. As the infection progressed, spots became enlarged, sunken, reddish brown to black in colour. On the thirteenth day, the lesions become necrotic and turned into dark brown in colour. The fungi were re-isolated from the infected leaves and were compared with the original culture of *F. fusaroides* and *F. moniliforme*.

Fusarium solani was pathogenic in glass house conditions and the symptoms of root rot infection recorded during the pathogenicity test were similar to the natural symptoms.

The rotting initially started at the root tip region and proceeded towards its distal portion. Gradually the root tissue became soft resulted in initiation of rotting of the root system. After 50 days symptoms spread towards the distal portion of roots, which resulted in total rotting of the root system.

DISCUSSION

The genus *Fusarium* is one of the largest fungal groups known to cause number of diseases on an extraordinary range of host plants. There are 1496 records of *Fusarium* found as both saprophytic as well as parasitic on host plants across the globe (Index Fungorum 2017). These fungi causes great economic loss across the globe, due to infecting the various medicinal and horticultural crops (Lecomte et al. 2016). This genus is known to produce mycotoxins like fumonisins and trichothecenes in their infected hosts and substrates on which they grow and cause toxic effects for consumers.

The infection of *Fusarium* on *A. vera* plants is of great concern as the plants are used in number of medicines and cosmetics.

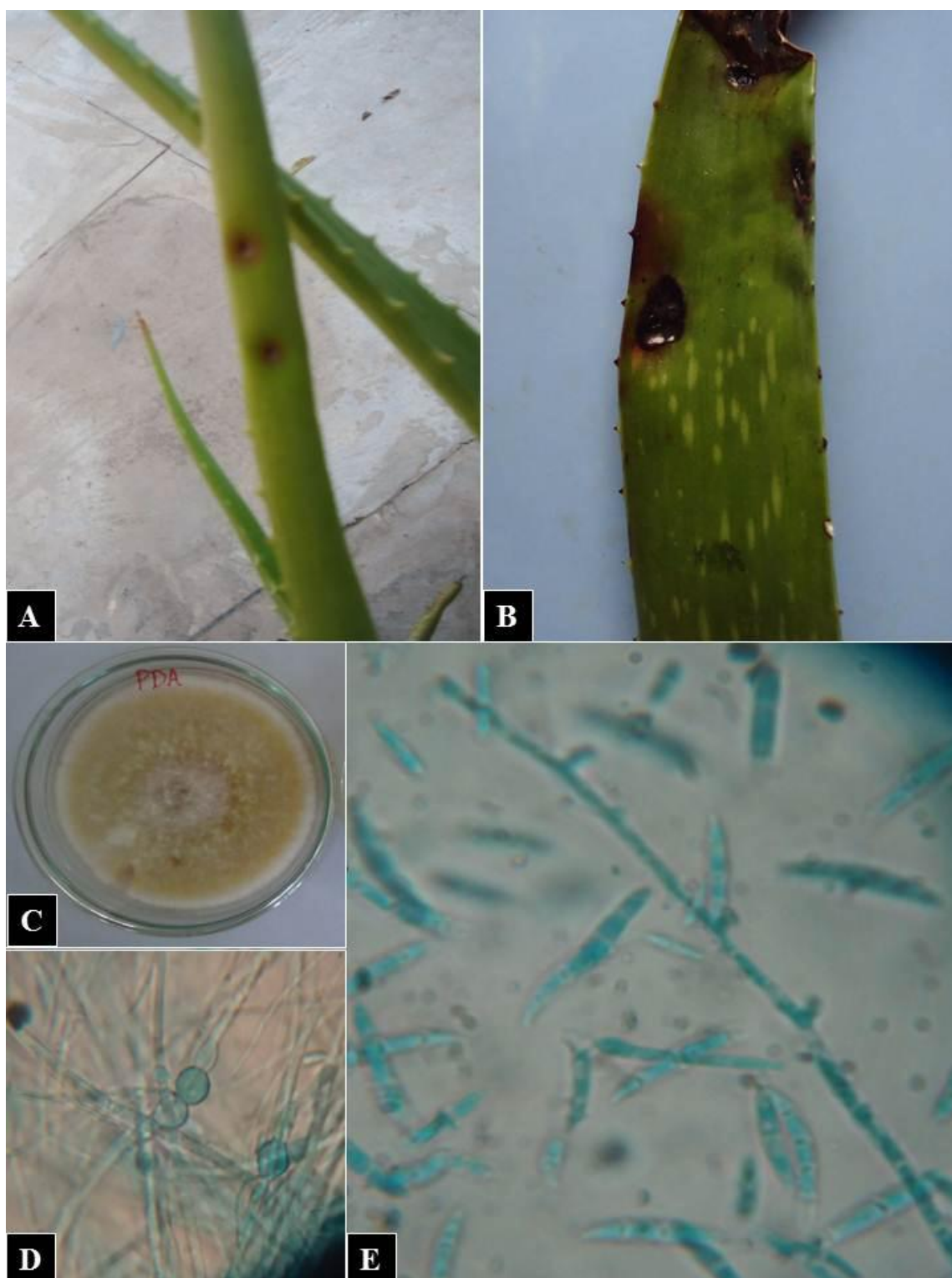


Fig. 1. Symptoms of leaf spots on *Aloe vera* caused by *Fusarium fusaroides*: **a & b)** Disease spot on leaves, **c)** culture on PDA, **d)** Macroconidia, **e)** chlamydospores.

Three species of *Fusarium* i.e. *Fusarium fusaroides*, *F. moniliforme* and *F. solani* were isolated from *A. vera* causing leaf spot and root rot diseases in plant. Previously, *F. fusaroides* was reported to cause pod rot in Groundnut (Subrahmanyam et al, 1980), leaf spot disease in pomegranate (Sherkar & Utikar 1982), leaf spot disease in maize (Bai et al. 1988)

and Pokkah boeng disease in sugarcane (Vishwakarma et al. 2016). Similarly, *F. moniliforme* has been previously reported as pathogen from various plant hosts including: malformation in mango (Summanwar et al. 1966), banana fruit rot (Khanna & Chandra 1976), Wilt of sunflower (Bhargava et al. 1978), sorghum (Raju et

al. 1999), stalk rot of maize (Bohra et al. 2001), foliage infection of *Dieffenbachia picta* (Palmucci & Barreto 2007), fruit rot of *Praecitrullus fistulosus* (Sankar et al. 2011), post flowering stalk

rots of maize (Kaur & Mohan 2012; Musmade et al. 2013; Ramesha & Krishna Naik 2017), foot rot disease on fenugreek (Singh et al. 2014) and Sugarcane leaf binding disease (Tiwari et al. 2016).



Fig. 2. Symptoms of leaf spots on *Aloe vera* caused by *Fusarium moniliforme*: **a & c**) Disease spots on leaves, **c**) culture on PDA (Adaxial and abaxial side), **d**) Macroconidia.

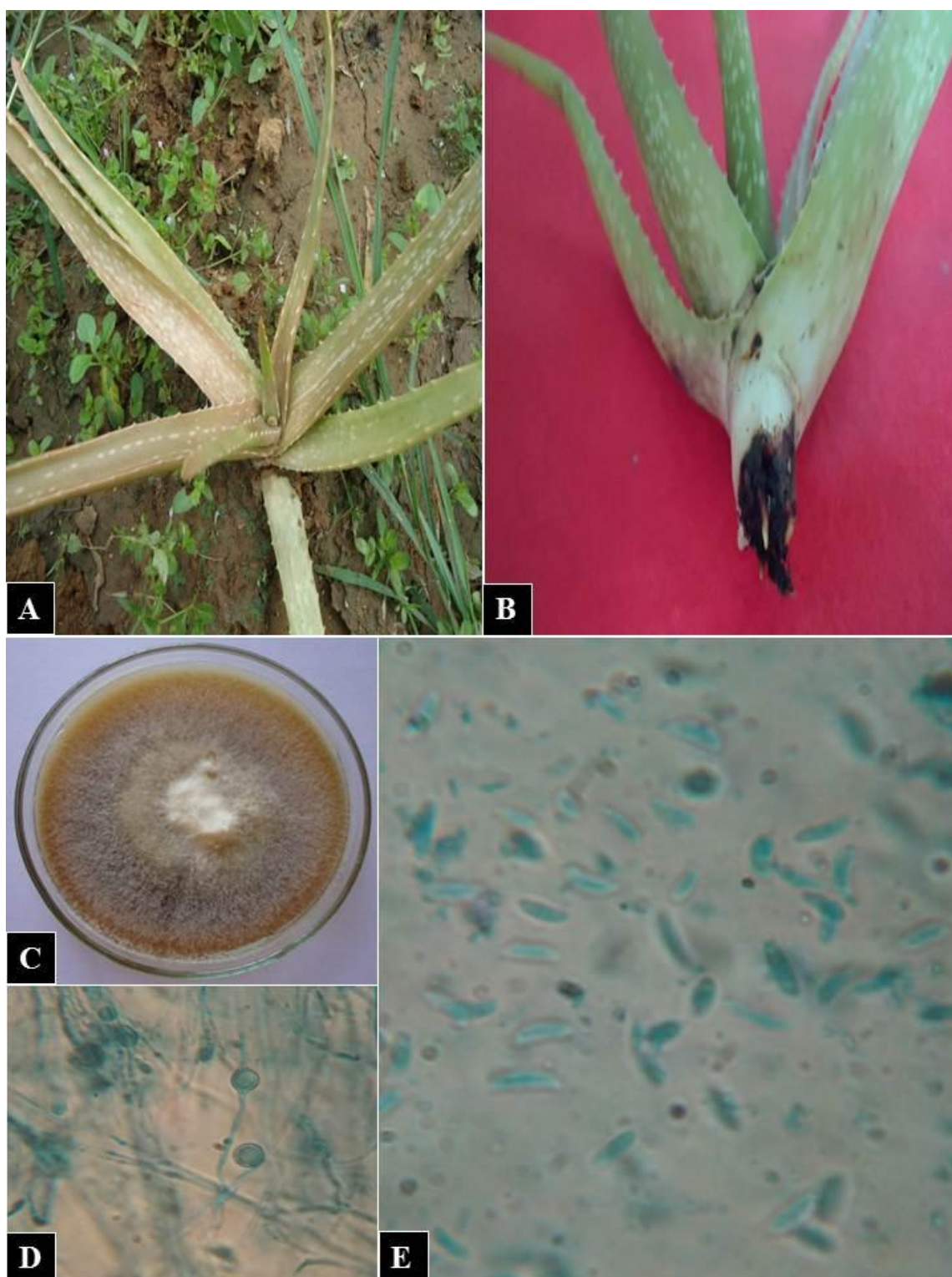


Fig. 3. Disease symptoms on *Aloe vera* leaves and roots caused by *Fusarium solani*: **a & b**) root rot, **c**) culture on PDA, **d**) Macroconidia, **e**) chlamydospores.

Root rot disease of *A. vera* caused by *F. solani* has been reported from Rajasthan (Sharma & Samota 2007; Jat & Ahir 2014), Madhya Pradesh (Bairwa 2008; Lal et al. 2016) India and China (Ji et al. 2007). Other diseases reported to cause by *F. solani* in India are: root rot of pea (Sen et al. 1970), rot of tomato (Pradeep & Gupta 1979), root rot of fennel

(Gupta & Strivastava 1976), root rot of guar (Satyaprasad & Ramarao 1981), root rot of *Salvia officinalis* (Mallesh & Narendrappa 2009), *Hedera canariensis* wilt (Mehraj et al. 2009), basal plate rot of onion (Bayraktar 2010), leaf spot of *Withania somnifera* (Chavan & Korekar 2011), root rot of *Zantedeschia ellitona* (Shanmugam et al. 2015), seedling blight of cucumber (Shanmugam et al.

2016) and necrosis, drying, black spots of cashew nut flowers (Wonni et al. 2017).

Some pathogenic fungi have been found to infect aloe plant such as *Fusarium phyllophilum* (Kishi et al. 1999), *Colletotrichum gloeosporioides* (Avasthi et al. 2011), *Fusarium oxysporum* (Chavan & Korekar 2011; Kawuri et al. 2012; Ilondu 2013), *Nigrospora oyrzae* (Zhai et al. 2013), *Phoma betae* (Avasthi et al. 2013), *Alternaria alternata* (Gupta & Masood 2003; Kamalakannan et al. 2008; Abkhoo & Sabbagh 2014), *Sphaeropsis sapinea* (Kamil et al. 2014), *Curvularia lunata* and *C. ovoidea* (Avasthi et al. 2015), *Alternaria tenuissima* (Vakalounakis et al. 2015) *Cladosporium sphaerospermum* (Avasthi et al. 2016a), *Phomopsis* sp. (Avasthi et al. 2016b), *Polyrostrata indica* (Avasthi et al. 2017a) and *Phoma eupyrena* (Avasthi et al. 2017b). Although, *F. solani* has already been reported on *A. vera* as root rot pathogen from Jaipur (Rajasthan), and as leaf spot pathogen from Jabalpur (Madhya Pradesh), India. However, there are no reports available for *F. moniliforme* and *Fusarium fusaroides*. This seems to be the first report of leaf spot disease on *A. vera* caused by *F. fusaroides* and *F. moniliforme* while second report of root rot by *F. solani* from India.

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