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Pathogenicity of mycoflora of tip-rot disease of *Aloe vera* (Syn *Aloe barbadensis* Miller), a common medicinal plant In Abraka, Delta State, Nigeria

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The mycoflora associated with tip-rot disease of *Aloe vera* (Syn, *Aloe barbadensis* Miller) and their pathogenicity was investigated in Abraka, Delta State. Fungi associated with diseased plants were isolated and identified as *Mucor hiemalis*, *Fusarium oxysporium, Pestalotia psidii, Cladosporium herbarum and Heteropatella alpina,* with occurrences by 46.22, 20.17, 13.45, 10.92 and 9.24%, respectively. Except *M. hiemalis*, all these fungi were pathogenic to *A. vera* and caused mild to severe symptom 14 days after artificial inoculation on healthy plants with mycelia fragments of the fungi. *F. oxysporium* and *P. psidii* were more aggressive in symptom induction than the other fungi. The significance of these findings has great implication on the reduction of aesthetic and therapeutic values of *A. vera* plant.

Key words: Pathogenicity, mycoflora, tip-rot disease, *Aloe vera.*

INTRODUCTION

Plants continue to be a major source of drugs and natural products as the basis of their therapeutics (Oyewale and Audu, 2007). Traditional medicine has advantage in the rural areas of developing countries, like Nigeria over modern ones because it is cheaper and easily accessible to most of the population (Edema et al., 2007). The genus *Aloe* L. is a member of the Liliaceae family. *Aloe* is derived from the Arabic word "Alloeh" which means "shinning bitter substances" (Akinyele and Odiyi, 2007). According to Adodo (2009), over 325 species of the genus *Aloe* have been identified but *Aloe vera* (Syn. *Aloe barbadensis Miller*) (Plate 1) is more popular all over the world because it propagates itself faster than any other known species. Hence *A. vera* is more readily available for use than any other species of *Aloe.*

It belongs to a large class of plants known as "Xeroids" because it possesses the ability to close its stomata completely to avoid loss of water. This makes the plant to have natural ability to survive long period of drought (Kafaru, 1994). Apart from its use as ornamental in homes, gardens and yards (Ilondu, 2011), *A. vera* is one of the oldest known medicinal plants with a long and illustrious history dating back to pre-biblical times (Olusegun, 2000). Biblical reports in John 19:39-40 reveals that it was used to embalm the body of our Saviour Jesus Christ as was the burial custom of the Jews. There is also a report that *A. vera* was an important part of the beauty regime of the Egyptian Queens: Nefertili and Cleopatra; and that Alexander the Great was convinced by Aristotle to take control of the Island of Socotra in Indian Ocean in order to possess their abundance of *A. vera* needed to heal his wounded soldiers (Daodu, 2000).

For more than 3500 years, healers and physicians have touted the benefits of *A. vera* (Mindell, 2006). *A. vera* contains over seventy-five nutrients and twenty

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Plate 1. A healthy *A. vera* plant.

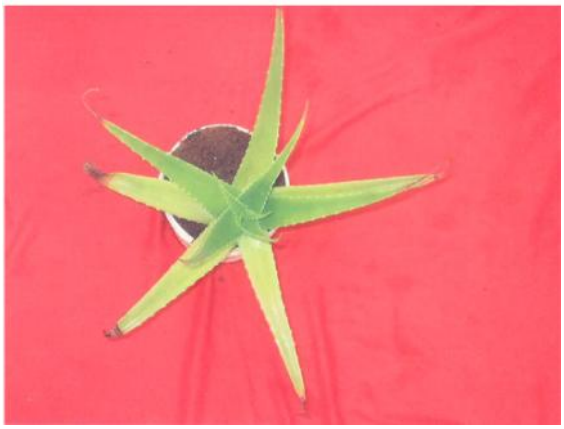

Plate 2. *A. vera* plant naturally infected with tip-rot disease.

minerals, nineteen amino acids and twelve vitamins. It is a pharmaceutical plant. It is also a source of many phytochemicals (Ilondu, 2011). In different parts of the world today, multibillion pharmaceutical and cosmetic industries utilize *A. vera* either solely or in combination with other materials for the production of diverse products that are of immense benefit to man (Udengwu and Arukwe, 2010). *A. vera* gel has been used as a natural remedy to sooth the pains of burns, rashes, insect bites and other skin irritations (Kafaru, 1994; Hegger, 1996; Olusegun, 2000); to cure ringworm and eczema (Ilondu and Okoegwale, 2002), for treatment of constipation, piles, impotence, liver and kidney problems, jaundice and ameneorrhea (Daodu, 2000; Talmadge et al., 2004; Steenkamp and Stewart, 2007); and has a modulating effect on human immunodefiency virus- HIV (Adodo, 2009). The antiinflamatory (Vazquiz et al., 2006) and antioxidant (Chandan et al., 2007; Kadiri, 2011) potentials of *A. vera* have been established.

A. vera is commonly referred to as “miracle plant” for its numerous uses particularly in the area of man’s health (Daodu, 2000). In Abraka community, many people now grow *A. vera* around their houses and herbal drinks are being formulated from the plant with either water or alcohol (Ilondu, 2011) for maintenance of good health. Two major diseases of *A. vera* which are prevalent in home gardens in Abraka are base and tip rots of the succulent leaves. This is a very serious limiting factor to the quality and quantity of leaves of *A. vera* plants. Under severe infections, the leaves drop and may lead to partial or complete defoliation of the plant depending on the severity of infection. Sometimes, severe infection may lead to premature death of the plant. Although, Ayodele and Ilondu (2008) have reported on the base-rot disease of *A. vera.*, information is lacking on the tip-rot disease of this miracle healing plant (Plate 2).

This study was therefore undertaken to provide information on the pathogenicity of the mycoflora associated with the tip-rot disease of *A. vera* in Abraka. It is hoped that the knowledge of the etiological agents will be a great prelude to formulating an effective control measure for the disease

MATERIALS AND METHODS

Collection of diseased *A. vera* leaves

Samples of *A. vera* leaves showing tip-rot disease symptoms were collected from a home garden in Bembo area, Ekrejeta village, Abraka. Abraka (Ethiope East Local Government Area) lies within latitude 05°47`N and longitude 06°6`E of Equator with an annual rainfall of 3,097.8 mm, annual relative humidity of 83% and annual mean temperature of 30.6°C (Efe and Aruegodore, 2003). The samples were kept in sterile paper bags and taken to the laboratory for isolation studies.

Isolation and identification of fungi from diseased leaves

A total of ten randomly selected diseased leaves from different *A. vera* plant were taken to the laboratory for isolation of the associate mycoflora. The method of Ilondu and Ayodele (2003) was adopted. The affected leaves were excised with sterile razor blade (4 mm long and five pieces per leaf), surface-sterilized for 2 min in 2% aqueous solution of sodium hypochloride and rinsed in two changes of sterile distilled water. The disinfected tissue pieces were blotted between sterile Whatman No.1 filter papers and aseptically plated randomly in 9 cm diameter Petri-dishes containing Potato dextrose agar (PDA) medium (3 pieces per plate). The cultures were incubated at room temperature of 28 ± 2°C on a laboratory bench for 3 to 5 days. The mycelia growing from the tissues were transferred onto fresh PDA medium amended with 1.0 mg/ml chloramphenicol and sub-cultured repeatedly until pure cultures of the isolates were obtained. Identification of isolates was done using light microscope and standard mycological manuals by Barnett and Hunter (1999) and Alexopoulus et al. (2002). Percentage frequencies of isolation (PFI) of all fungi were calculated using the formula:

Table 1. Fungi isolated from tip-rot disease of *A. vera* and their frequency of isolation.

Fungi isolated	Total number of times isolated	Isolation frequency (%)
Mucor hiemalis	55	46.22
Fusarium oxysporium	24	20.17
Pestalotia psidii	16	13.45
Cladosporium herbarum	13	10.92
Heteropatella alpina	11	9.24

Plate 3. Artificially inoculated *A. vera* plants.

$$PFI = \frac{\text{No. of times a fungus is encountered}}{\text{Total no. of times all fungus were encountered}} \times \frac{100}{1}$$

Pathogenicity of Isolates

Top garden soil of 0 to 20 cm in depth collected from Department of Agricultural Education Teaching and Research Farm, Delta State University, Abraka was used for the pathogenicity test. The soil was collected in 45 × 38 × 0.4 cm black polythene bags and sterilized by autoclaving at 121°C for 1 h, then repeated after 24 h for 1 h and left to cool for 2 days before use. Young suckers obtained from healthy *A. vera* plants in home garden in Bembo area were planted in the polythene bags each containing 5 kg of sterile soil and maintained in a screen house under prevailing conditions of temperature (28 ± 2°C) and light for three weeks before inoculation.

The inoculation of the test plants was carried out according to the technique of Ayodele and Ilondu (2008). Colonies of isolates were incubated in PDA for 10 days on the laboratory bench at room temperature (28 ± 2°C). The leaves of healthy *A. vera* plants were surface sterilized with 70% alcohol. Two plants were used for each fungal isolate and the tip of the four lower leaves on each plant received needle-jab punction made in four different places. A sterile inoculating needle was used to remove mycelial disc (2 mm) from pure culture of each isolate and were individually inoculated onto the wounded leaves. Plants inoculated with sterile PDA discs served as control. Both inoculated and control plants were properly labelled and examined daily for symptom development up to 14 days after inoculation. The degree of rot caused by each isolate was assessed based on disease severity rating. Photographs of tip rot symptoms produced by artificial inoculation were taken to compare with those observed on naturally infected leaves collected from the *A. vera* garden. The fungi were re-isolated from the inoculated diseased leaves and cultured in PDA plates. The morphology of each pathogenic fungus was compared with that of original cultures.

RESULTS AND DISCUSSION

The fungi isolated across all the diseased leaves pieces of *A. vera* were *Mucor hiemalis* wehmer, *Fasarium oxysporium* Schlecht*, Pestalotia psidii* Pat.*, Cladosporium herbarum* link ex Fr. and *Heteropatella alpina* Ellis & Everh (Table 1). The pathogenicity tests with these fungi showed that initial disease symptom were visible as water-soaked lesions and were well-developed by 2 weeks after inoculation. The rot appeared chlorotic at first but later turned brown and shriveled (Plate 3). This is the first report on the fungal, tip-rot disease of *A. vera* in Abraka, Nigeria.

Plants inoculated with *F. oxysporium* and *P. psidii* showed complete and severe tip-rot symptoms, respectively. Similarly, plants inoculated with *C. herbarum* showed mild infections, while *H. alpina* showed moderate infections. No tip-rot symptoms were observed in the control plants and those inoculated with *M. hiemalis* (Table 2). The pathogenicity was in the order of *F. oxysporium* > *P. psidii* > *H. alpine* > *C. herbarum.* The spore of these fungi may have come in contact with the *A. vera* leaves by air movement as reported by Domsch and Gams (1972). *Cladosporium*, *Fusarium* and *Mucor* species were among the fungal airspora of Abraka (Dongo and Ayodele, 1997).

C. herbarum has been reported to be the most common fungus on dying and dead plant substances (Domsch and Gams, 1972), while *H. alpina* can be parasitic or saprophytic (Barnett and Hunter, 1999). *M. hiemalis* was not found to be pathogenic in the inoculation test. Possibly, it acted as a secondary invader of the tip-rot lesions caused by the pathogenic isolates. Thus, as pointed out by Ilondu and Ayodele (2003), such organism when tested against the host in the absence of the primary pathogen would have no effect. Pathogenic fungi on the leaf surface may provide nutrient directly for the saprophytes either through decay of the tissue or liberation of nutrients from their teleospores (Ilondu and Ayodele, 2005). This may probably be responsible for the highest isolation frequency of *M. hiemalis* in the study. Domsch and Gams (1972) also reported that the list of

Table 2. Symptom induction and re-isolation of fungi isolates from *A. vera* leaves 2 weeks after inoculation.

Inoculation	Re-isolation from leaf tissue 2 weeks after inoculation	Disease rating
Cladosporium herbarum	+ve	+
Fusarium oxysporium	+ve	++++
Heteropatella alpine	+ve	++
Mucor hiemalis	-ve	--
Pestalotia psidii	+ve	+++
Contol	-ve	--

Disease rating: -- = no infection (0%); + = mild infection (10-30%); ++ = moderate infection (31-60%); +++ = severe infection (61-90%); ++++ = complete infection (100%); +ve = fungi re-isolated; -ve = fungi not re-isolated.

dead substrate colonzied by *M. hiemalis* is very extensive that it does not allow any particular requirement of the fungus to be recognized.

The most virulent fungus causing tip rot disease of *A. vera* was *F. oxysporium* and was followed by *P. psidii. F. oxysporium* have been reported to be an aetiological agent of many crops (Osuinde and Ikediugwu, 1996; Ilondu et al., 2010; Maranzu and Wokocha, 2010) and major causes of mycotoxicoses in man and animals (Rai and Acharya, 1999; Matthias et al., 2003). It has been reported as a pathogen of *A. vera* (Ayodele and Ilondu, 2008). *Pestalotia* sp. have been proven to be pathogenic on leaves of date palm (*Phoenix dactyllfera* L.) (Eziashi et al., 2008) and *Rhododendron* spp. (Barnett and Hunter, 1999).

Conclusion

The presence of these fungal pathogens in *A. vera* plants has great implications on the health of the rural populace that use this plant in multi-purpose herbal preparations and recipes to treat various ailments. This is because these pathogens could produce mycotoxins in the infected hosts which are hazardous to human health when consumed. A case of mycotoxin production in *Fusarium* spp. has been reported (Matthias et al., 2003). Besides, the aesthetic value of *A. vera* is at stake. In view of this, work is in progress in developing an ecofriendly control measure for this disease with extracts of three *Jatropha* spp. Consequently, the medicinal and aesthetic value of *A. vera* will continually be maintained.

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