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ISOLATION AND IDENTIFICTION OF FUNGI INFECTING *ALOE VERA* PLANT

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ABSTRACT

This study included isolation and identification of the fungi associated with *Aloe vera* (L.) in nurseries and plant gardens. The results showed that the fungi *Alternaria alternata, Fusarium oxysporum, Fusarium solani, Nigrospora oryzae, Cladosporium herbarum, Stemphylium botryosum, Aspergillus niger, Penicillium* sp. were isolated from the diseased leaves of *Aloe vera* showing spots and blight symptoms. The percentages of disease incidence in March, Jun and August were found to be 5, 50 and 60 %, respectively. Pathogenicity test of *Alternaria alternata, Fusarium oxysporum, Nigrospora oryzae* and *Cladosporium herbarum* showed that disease index were 50, 25,25and 12.5 %, respectively. The fungi isolated on *Aloe vera* in this study have not been published previously in Iraq.

Key words: *Aloe vera*, Fungi, Gardens, Isolation, Nurseries.

INTRODUCTION

Aloe vera (L.) Burn.f belongs to family Aloaceae, is perennial, succulent plant with a height of 60-100 cm,stemless sessile herb, leaves 30–50 cm long and 10cm broad, color pea-green bright yellow tubular, flowers arranged in a slender loose spike (Youngken, 1950). Four hundred species of *Aloe* have been reported worldwide, *A. vera* is the most extensively cultivated as a medicinal plant. It is adapted widely all over the world, in the tropics to temperate regions especially. Aloe leaves are filled with gel which is the most important constituent of the plant and has great medicinal value. *A. vera* has been reported to contain amino acids, anthraquinones, enzymes, lignin, minerals, mono and polysaccharides, salicylic acid, saponins, sterols and vitamins (Barcroft and Myskja, 2003). *A. vera* plants have been entered in many herbal drugs for the keeping of good health. In cosmetic industries Aloe is used in the production of soap for bathing, shampoo, hair wash, toothpaste and body creams (Daodu, 2000). *A. vera* gel has been reported very effective for the treatment of sore and wounds, skin cancer, skin disease, cold and cough, constipation, piles, fungal infection, asthma, ulcer and diabetes (Daodu, 2000; Djeraba and Quere, 2000; Olusegun, 2000; Davis and Moro, 1989). *Aloe vera* has a long history of popular and traditional use. It is used in traditional Indian medicine for constipation, colic, skin diseases, worm infestation, and infections .In Chinese medicine, it is often recommended in the treatment of fungal diseases (Heber, 2007). A total of 15 fungi, including *Fusrium roseum, Fusarium oxysporum, Alternaria alternate, Alternaria dianthi, Aspegillus niger, Aspergillus fumigatus, Drechslera australiensis, Curvularia senegalensis, Colletotrichum dematium, Nigrospora oryza,*and *Trichoderma viride* were isolated from the leaves samples of *A.vera* collected from different areas(Singh *et al*., 2014).Surveys have shown that the common disease on the *A.vera* plants was leaves spots which causes harmful effects on the medical value of the plant parts, and

other fungi *Fusarium solani, Aspergillus niger, Penicillium* spp. were recorded (Chavan and Korekar, 2011). The purpose of this study is to identify the pathogenic fungi infecting *Aloe vera* plants in some nurseries in the Baghdad area.

MATERIALS AND METHODS

Isolation: *Aleo vera* infected leaves showing ,were collected from the Department of Drugs and Medicinal Plants, Pharmacy College - University of Baghdad and some nurseries, the leaves were washed with water, cut off to parts of 2-5 mm length, surface sterilized in 1.5% sodium hypochlorite solution for (1-2) minutes, rinsed with sterile water and then cultured on Potato Dextrose Agar (4 pieces/plate) and incubated at 27±2°C. Fungal growth was purified by the single hyphal tip method and subculture on PDA to serve as inoculum source.

Identification of the pathogen: The identification of fungi was made by preparing slides of the fungal growth and observing them under a compound microscope. Pure cultures of fungi were prepared and maintained on potato dextrose agar (PDA) slants.

The fungi mounted on slides, stained with lacto phenol-cotton blue and examined under microscope diagnosis based on morphological characteristics of the colonies and spore and preserved in slants at 4-5 C°.

Estimation the disease incidence: The disease percentage was reported from surveys, visiting the plant garden and the nurseries cultivated with *Aloe vera* plants and examined the infected plants twice in onset and the end of the season. One of the main symptoms that appeared on the diseased plants include a small superficial necrosis, brown spots on leaves, Sometimes these spots connect together and cause to dying the leaf entirely and become deepened and more darker under wet conditions, and blight seem on the tip and edges of the leaves or all the leaf, and sometimes appears white color on spotting area resulting from the fungus mycelium, and rotting the basis of the leaves near the soil, which cause the death of the little seedlings as all or death many of leaves in old seedlings (Fig. 1, 2, 3) which is similar to the symptoms mentioned by Kawuri *et al*. (2012). The percentage of disease incidence was estimated by using the following formula:

$$\text{The percentage of disease incidence} = \frac{\text{No. of diseased plants}}{\text{No. of all plants}} \times 100$$

The pathogenicity test: Based on Koch postulate Pathogenicity studies was conducted on healthy leaves of *Aloe vera* plants, cleaned spotless of similar size leaves were collected, washed with tap water and placed on the surface of wet-cotton in a petri dish. Inoculums plug (5 mm diam.) of *Alternaria alternata*, *Fusarium oxysporum*, *Nigrospora oryzae*, *Cladosporium herbarum* cultures were placed on the leaves. Plugs of PDA only were placed on another leaves for control. Three replications of each treatment were used. The plates were maintained at room temperature until the development of symptoms the data had been recorded, and the treated specimens were ranked on the basis of the disease severity and assessed as follows: 0- leaves healthy, 1- a few spots or slight necrosis as (1-25%) from leaf size, 2- the spotting take over (26-50%), 3- the spotting take over (51-75%), 4- blighting takes over (76-100%) (El-Morsy, 2004).

$$\text{Disease index (DI)} = \frac{no.leaves\ in\ degree0\times0\ldots..no.leaves\ in\ degree4\times4}{No.\ of\ leave\ all\ degree\ \times max.degree\ of\ infection} \times 100$$

RESULTS AND DISCUSSION

Identification: Results of isolation and identification depending on the keys of each fungus and on the shape of conidia and conidiophores that formed on the fungal growth on PDA (Booth, 1971; Ellis, 1971; Barnett and Hunter, 1972), revealed that the fungi *Alternaria alternata*, *Fusarium oxysporum*, *Fusarium solani*, *Nigrospora oryzae*, *Cladosporium herbarum*, *Stemphylium botryosum*, *Aspergillus niger*, *Penicillium* sp. were found associated with the infected leaves of *Aleo vera*.

Disease incidence: The results in table (1) showed that the disease incidence during March, June and August months were estimated to be 5, 50, and 60 %, respectively. The injury on the plants is more common in nurseries and became more clear and severe in climatic conditions appropriate to infection, high temperature in the atmosphere associated with high humidity in the soil. Significant variation in the ability of fungi to cause the disease between June, September and August were observed. Similar results concerning the disease incidence and injury were reported in West Bengal State throughout the year from January to December caused by *Alternaria brassicae* with infection percentages 83.28% - 95.71% (Ghosh and Banerjee, 2014).

Table (1): Disease percent on *Aloe vera* leaves nurseries.

The months	Disease percent %
March	5
Jun	50
August	60

The pathogenicity test: The results in table (2) indicated that the disease index of the fungi *Alternaria alternata*, *Fusarium oxysporum*, *Nigrospora oryzae* and *Cladosporium herbarum* were estimated to be 50, 25, 25,12.5 %,respectively, compared with 0% in control. Leaf spots, similar to those observed on infected *Aleo vera* in the nursery were manifested on the leave is used in the test after 20 days of inoculation. The same fungal isolates were re-isolated from the inoculated leaves of *A. vera*. It was reported that among many fungi recorded to infect *A. vera*, *F. oxysporum* was fond the most dominant that representing 90 %, followed by *Drechslera australiensis* 73.3% *Trichoderma viride* 66.6% and *Alternaria alternate* 33.3% (Singh *et al*., 2014). Another study Ilondu (2013) showed that *F. oxysporum* was the more virulent and followed by *Pestalotia psidii* and *Cladosporium herbarum*. Several previous studies reported similar results concerning the pathogenic fungi infecting *Aleo vera* plant in the world as leaf spots on *Aloe vera c*aused by *Nigrospora oryzae* in china was recorded by Zhai *et al.* (2013). Fungi associated with the base rot disease of *Aloe vera* (*Aloe barbadensis*) were investigated by Ayodele and Ilondu (2008) in Nigeria and found the percentage frequency were as *Aspergillus verocosa* 28.03%, *Fusarium oxysporium* 24.24% and *Torula herbarium* 15.15%.

Table (2): Disease index of pathogenicity test on *Aloe vera* leaves *in vitro* condition.

Species	(DI) %
Alternaria alternata	50
Cladosporium herbarum	25
Fusarium oxysporum	25
Nigrospora oryzae	12.5
control	0.0

Figure (1): Symptoms of yellowing, browning on *Aloe vera* leaves surfaces and bases near the soil, then turn to blights causing rapid death to entire leaves.

Figure (2): Symptom of necrotic tissue formed on *Aloe vera* leaves, the spots often are small and enlarge and deepen.

Figure (3): Symptoms of dark brown spots on leaves margins of *Aloe vera* and then turn to a large patch, quickly turn to brown blighting .

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عزل و تشخيص الفطريات التي تصيب نبات *Aloe vera*

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شملت الدراسة عزل وتشخيص المسببات الفطرية التي ترافق نبات الصَبر الحقيقي او الألَوَة الحقيقية (L.) *Aloe vera* في المشاتل والحدائق النباتية، و اظهرت النتائج بان الفطريات
Fusarium oxysporum, Fusarium solani, Nigrospora oryzae, Cladosporium herbarum, Stemphylium botryosum, Aspergillus niger, Penicillium sp.
هي التي عزلت من الأوراق المصابة والتي عليها أعراض التبقع و اللفحة. وكانت النسبة المئوية للاصابة في أشهر آذاروحزيران وآب (٥، ٥٠، ٦٠٪) على التوالي. وأظهرت نتائج اختبار القدرة الامراضية لكل من الفطريات
Alternaria alternata, Fusarium oxysporum, Nigrospora oryzae, Cladosporium herbarum
وان نسبة الدليل المرضي كانت ٥٠، ٢٥،٢٥ ، ١٢,٥٪، على التوالي. ان الفطريات التي عزلت من نبات *Aloe vera* في هذه الدراسة لم تنشر سابقا في العراق.