Bull. Fac. Agric., Cairo Univ., 60:342-350 (2009)

MYCOFLORA ASSOCIATED WITH *Acacia saligna* (Labill.) Wendl. THE DOMINANT PLANT SPECIES IN AL-AHRASH PROTECTORATE- RAFAH-NORTH SINAI- EGYPT

(Received:24.3.2009)

By
K.F. El-Sahhar, A. M. Shaltout *and L. A. Qasem**

*Department of Agricultural Botany, Faculty of Agriculture, Cairo University, Giza, Egypt,*Plant Pathology Research Institute, Agricultural Research Centre, Giza, Egypt** and Environment and Forestry Department, Faculty of Agriculture, Damascus University, Syria.*

ABSTRACT

The present study deals with fungi existing in Al-Ahrash protectorate, an area of 8 km^2, 370km eastern to Cairo, in Egyptian Rafah, North Sinai Governorate (31^o $18^/$ N - 34^o $14^/$ E) which was declared a developing resources protected area according to the Prime Ministerial Decrees 1429/ 1985 and 3379 / 1996.

Results include a survey and identification of mycoflora associated with both of the soil and shoot of *Acacia saligna* (Labill.) Wendl., the dominant plant species in Al-Ahrash protectorate.

Survey study of mycoflora associated with the soil surrounding roots of *Acacia saligna* revealed that *Fusarium solani* was dominant with frequency of 26.7 % ranging from 10 to 45 %. Twenty fungal species were isolated from soil of the studied area, nine of them were soil borne fungi and the remainder eleven might affect plant leaves. *F. solani* also recorded the highest frequency (35%) in the neighbouring cultivated area. Number of fungal species associated with *Acacia saligna* trees increased in mature s compared with juvenile plants.

Identification of VAM fungal spores extracted from soil of the studied area proved that they belong to genus *Glomus*. Average number of spores / 50g soil was 1230 with a range from 400 to 2200. This number decreased to 900 spores in soil of cultivated fields outside Al-Ahrash protectorate. On the contrary, the number increased to 7100 spores in soil surrounding roots of juvenile *Acacia saligna* plants.

Seven fungal species which may cause leaf spots to *Acacia saligna* were isolated from diseased phyllodes. Both *Botryodiplodia theobromae* and *Cladosporium herbarum* recorded an average occurrence of 22.5% with a range of 20-30%, being the highest in this respect.

Key words: *Acacia saligna (Labill.) Wendl., Al-Ahrash protectorate, Glomus, leaf spots, mycoflora, soil borne fungi and VAM fungi.*

1. INTRODUCTION

Al-Ahrash protectorate located north of Sinai Peninsula, 370 km eastern Cairo and covering an area of 8 km^2 has been declared developing resources protected area by the Prime Ministry Decree number 1429/1985 modified by the Decree number 3379 /1996. It occupies part of the sand dune system (up to 60 m.a.s.l.) between Al-Arish and Rafah cities, close to the Mediterranean shore and falls under its climatic influence (Anon., 2001).

The natural vegetation of Al-Ahrash protectorate is mainly composed of *Acacia saligna* (Labill.) Wendl. This tree serves as a source of pasture, wood and animal shelter for the local inhabitants, beside its role as sand dune fixer species (El-Sahhar *et al.,* 2009).

Previous investigations indicated that *Acacia saligna* is subjected to various fungal diseases; *e.g*., rust (Morris, 1987) and wilt (Roux *et al*., 2007) In addition, the association of mycorrhiza and bacteria with

Acacia saligna was studied. David and Schenck (1983) mentioned that chytridiaceous fungi (CF) have been observed on spores of vesicular- arbuscular mycorrhizal fungi (VAMF) and can limit their development in pot cultures and in the field. The fungicide finaminosulf reduced the percentage of azygospores of *Gigaspora margarita* with zoosporangia of CF.

Krishna and Dart (1984) tested six mycorrhizal fungi as inoculants for pearl millet grown in pots with sterile soil. Mycorrhizal inoculation increased dry matter and phosphorus uptake at levels less than 20kg/ ha. At higher P levels the mycorrhizal effect was decreased. These studies performed in sterilized soil suggest that inoculation of pearl millet with efficient VAM fungi could be extremely useful in P deficient soils. However, its practical utility depends on screening and isolation of fungal strains which perform efficiently in natural (unsterilized) field conditions.

Jasper *et al.* (1989) examined the hypothesis that hyphae of a vesicular-arbuscular mycorrhizal fungus in dry soil remain ineffective, but that the infectivity decreases if the soil is disturbed. They found that external hyphae of *Acaulospora laevis* Gerd. remained highly ineffective. However, disturbance of the dry soil containing the external hyphae severely reduced the infectivity of the hyphae. After fluorescent staining, fungal structures with fluorescing nuclei were readily visible both within and attached to the roots of *Acacia saligna* which had no fluorescing nuclei.

Benbrahim and Ismaili (2002) investigated the effects of single dual inoculation with *Rhizobium* and arbuscular mycorrhizal fungi (AMF) on *Acacia saligna* plants to increase nutrient uptake and plant growth. The substrate was a clay-loamy soil low in plant available N and P. The soil has been fumigated by using Basamid. Plants were either inoculated with an AMF alone, with a strain of a compatible *Rhizobium* alone, or with both endophytes. A control was uninoculated. All treatments received a N- and P- free nutrient solution. Plant growth, nodulation, mycorrhizal infection, nutrient content (N and P) and N_2-fixation were studied. Under the experimental conditions tested, dual inoculation significantly increased total dry weight by 56.9%, mycorrhizal infection 14- fold and N and P content of plants by 70.9% and 76.3%, respectively. *Rhizobium* inoculation alone significantly increased shoot dry weight, nodulation and total N content. Mycorrhizal inoculation increased root dry weight, mycorrhizal infection and total shoot P content.

Pretorius *et al.* (2003) tested extracts from 26 plant species representing 16 families, among them two species belong to the genus *Acacia;* namely, *A. erioloba* and *A. karoo* collected in the Free State Province of South Africa. Extracts were tested *in vitro* for their potential to inhibit the growth of eight plant pathogenic fungi and five plant pathogenic bacteria. None of the crude extracts showed any mycelial growth inhibition of the eight tested fungi. All of the extracts inhibited the growth of one or more of the five plant pathogenic tested bacteria but to varying degrees.

Roux *et al*. (2007) found that *Ceratocystis albifundus* causes the disease known as wattle wilt of non-native *Acacia mearnsii* trees in South Africa, Uganda and Kenya. Infection results in rapid wilt and death of susceptible trees and stem cankers on more tolerant trees.

As far as the authors are aware, no previous studies dealt with the mycoflora of Al-Ahrash protectorate. In this study, the identity and frequency of mycoflora found in the soil surrounding the root and on the phyllodes of *Acacia saligna*, the dominant and multipurpose plant species in the area of study are subjected to investigation. This study provides a pioneer survey of mycoflora in Al-Ahrash protectorate and will flourish the state - of - knowledge in this concern.

2. MATERIALS AND METHODS

Field expeditions were carried out to Al-Ahrash protectorate (31^{o} $18^{/}$ N - 34^{o} $14^{/}$ E) during spring season of 2008 (Fig. 1). *Acacia saligna* (Labill.) Wendl. proved to be the dominant plant species in this area. Details of sampling procedures were previously mentioned (El-Sahhar *et al.*, 2009).

2.1. Mycoflora of the soil surrounding the roots of *Acacia saligna*

In order to isolate the inhabitant mycoflora, ten soil samples, 20-40 cm from

soil surface, resembling the ten studied quadrates fixed at Al-Ahrash protectorate were collected from the soil surrounding roots of *Acacia saligna*, which have a heavy surface spreading root due to its vigorous root suckering ability. An additional soil sample was collected from outside Al-Ahrash protectorate resembling the neighbouring area cultivated with barley to spot any difference in soil mycoflora due to bedouin activity. Moreover, a soil sample surrounding the roots of juvenile *Acacia saligna* was also taken to be compared with the previous samples. This might elucidate any alteration in soil mycoflora due to existence and growth of *Acacia saligna* in this area.

Fig. (1): A satellite imagery of Al-Ahrash protectorate, Rafah, North Sinai,Egypt (c.f. Google Earth web site , labelled and studied area was located).

2.1.1. Fungi

The isolation method of Pierr and Francis (2000) was followed. A soil sample, 10 g, of each of the collected samples was transferred to a flask containing 90 ml sterilized water and vigorously agitated for 30 min. Serial dilutions of soil suspension 1:10, 1:100, 1:1000 and 1: 10000 were carried out. One ml. of the latter suspension was placed in a sterilized Petri dish before pouring Martin's medium composed of 10 g glucose, 5 g peptone, 1 g potassium dihydrogen phosphate, 0.5 g magnesium sulphate, 15 g agar, 0.03 g rose Bengal, 0.03 g streptomycin and 1000 ml distilled water (Martin, 1950). The dishes were shaken gently to spread the soil suspension allover the medium. Dishes were incubated at 25± 2 ^{o}C for 5 days. Growing fungal colonies were transferred onto Petri dishes contained PDA medium which is composed of 200 g peeled and sliced potato, 17g agar, 20g dextrose and 1000 ml distilled water (Riker and Riker, 1936). Cultures of single spores or hyphal tips were secured for further studies.

2.1.2. Mycorrhizal fungi

Vesicular-arbuscular mycorrhizal (VAM) fungi in collected soil samples were investigated. A representative sample (50 g) of each studied soil was placed in a polyethylene bag. Extraction of VAM propagules in the soil was carried out under laboratory conditions as soon as possible using wet-sieving and decanting technique (Gerdemann and Nicolson, 1963) as follows:

i. A sample (50 g) of each soil was mixed with 1000 ml water and stirred carefully by a stick until particles were

loosen before being left for a few seconds to allow the heavier particles to settle down.

ii. The supernatant was then allowed to pass through soil sieves. First sieve (500 μm- hole-diam.) to remove the large pieces of organic matter, followed by a fine one (64 μm) to catch the small particles of soil which include the VAM spores.

iii. These particles were washed in a stream of tap water, then driven to a beaker, suspended in water and adjusted to a known volume (100 ml).

iv. One ml of the spore suspension was transferred into a Petri dish (9 cm diam.) containing a filter paper (Whatman No. 1,7 cm diam.) marked with small squares (1x1 cm) to score the number of spores using a dissecting binocular microscope.

Purified VAM spores were identified according to the morphological characteristics described by Gerdemann and Trappe (1974), Trappe (1982) and Trappe and Schenck (1982).

2.2. Mycoflora of *Acacia saligna* **phyllodes**

Phyllodes (modified flat leaf-like petioles, and no blade, since true leaves are absent) exhibited disease symptoms were carefully washed in tap water then cut into small pieces. Infected samples were surface sterilized in 0.5% sodium hypochlorite solution for 3 min. before being thoroughly rinsed in sterilized water and dried between two sterilized filter papers. The treated portions were transferred onto Petri dishes containing potato dextrose agar medium (PDA) amended with 0.01% streptomycin in order to minimize bacterial contamination. Plates were incubated at 25 ± 2 ^{o}C for 3 days. The developed fungi were carefully transferred to PDA slants and kept at 8^{o}C for further studies. Pure cultures were obtained from each isolate using the single spore or hyphal tip technique (Dhingra and Sinclair, 1985).

Fungi isolated from the soil surrounding roots and those from phyllodes were examined microscopically. Identification of the fungi was carried out according to descriptions given by Booth (1971), Domsch *et al*. (1980), Nelson *et al*. (1983), Barnett and Hunter (1986) and Sneh *et al.* (1991) at the Mycology Research and Plant Disease Survey Department, Plant Pathology Research Institute, Agricultural Research Centre, Giza, Egypt. Scientific names of fungi were revised according to the Annual Checklist of Bisby *et al.* (2009).

3. RESULTS AND DISCUSSION

Knowledge of fungi associated with *Acacia saligna* at Al-Ahrash protectorate either in the soil surrounding the roots or which affect the shoot is substantially incomplete, if not absent. Hence, the mycoflora found in both of the soil and the phyllodes were subjected to investigation in order to determine their identity and occurrence frequency.

3.1. Mycoflora of soil surrounding roots of *Acacia saligna*

3.1.1. Fungi

A survey study of mycoflora associated with the soil surrounding the roots of *Acacia saligna*, the dominant plant species at Al-Ahrash protectorate was performed to detect the main associated fungi in the soil of the studied area. Frequencies of fungi are given in Table (1).

With respect to the average frequency of fungi, *Fusarium solani* (Mart.) Sacc. came on the top with a frequency of 26.7%, and range from 10 to 45%. On the contrary, each of *Aspergillus ochraceus* G. Wilh., *Fusarium moniliforme* J. Sheld. and *Rhizoctonia solani* J.G. Kühn recorded a frequency of 0.5%, being the least in this concern, with a range of 0-5%.

Penicillium sp. showed a frequency of 19.5%, with a range from 10 to 35%, being the second after *Fusarium solani*. *Mucor* sp., however, came third in this respect with a frequency averaged 10.5% and a range from 20 to 30%. All the remainder fungi had an average less than 8% frequency with a range from 0 to 25%.

Referring to Farr *et al.* (1989) the fungi isolated from the soil of the studied area could be divided into two groups; the first being soil borne fungi and the second affect plant leaves. Fungi belonging to both groups were isolated from the soil of the study area and were more or less equal in number. The first group included fungi number: 7, 10 to 15, 18 and 20. Whereas, the second group included fungi number 1 to 6, 8, 9, 16, 17 and 19. (Table 1).

Regarding the soil sample collected from the cultivated area adjacent to Al-Ahrash protectorate to spot any variation in the

Table (1): Frequency (%) of fungi associated with the soil surrounding the roots of *Acacia saligna* at Al-Ahrash protectorate

No.	Fungi	Frequency (%) in quadrates										Average	Range	H.I.	J.A.
		1	2	3	4	5	6	7	8	9	10				
1	*Alternaria alternata* (Fr.) Keissl.	13	10	10	10						10	5.3	10-13	15	25
2	*Aspergillus clavatus* Desm.													5	
3	*Aspergillus flavus* Link		10	5								1.5	5-10		
4	*Aspergillus niger* Tiegh.			10				10	20			4.0	10-20	10	
5	*Aspergillus ochraceus* G. Wilh.									5		0.5	0-5		
6	*Chaetomium globosum* Kunze	20			5	5		10		10		5.0	5-20	5	
7	*Chonaephora* sp.		5						5	5	5	2.0	5-5	5	
8	*Cladosporium herbarum* (Pers.) Link		10									1.0	0-10	5	10
9	*Fusarium moniliforme* J. Sheld.							5				0.5	0-5		
10	*Fusarium oxysporum* Schltdl.			20	20	5	10	10				6.5	5-20	10	5
11	*Fusarium semitectum* Berk. & Ravenel		10						10			2.0	0-10		
12	*Fusarium solani (Mart.) Sacc.*	27	10		45	50	30	30	15	15	45	26.7	10-45	35	25
13	*Gonatobotrys simplex* Bonord.		10									1.0	0-10		
14	*Macrophomina phaseolina* (Tassi) Goid.								15		5	2.0	5-15		
15	*Mucor* sp.			20		25	30			30		10.5	20-30	10	
16	*Penicillium* sp.	20	10	35	20		30	10		35	35	19.5	10-35		25
17	*Pestalotia acaciae* Thüm.		15					10	10			3.5	10-15		
18	*Rhizoctonia solani* J.G. Kühn							5				0.5	0-5		
19	*Stemphylium botryosum* Sacc.	20	10			15		10	25			8.0	10-25		
20	*Ulocladium* sp.														10

Empty space = Not recorded.

$$\textbf{Freguency (\%)} = \frac{\text{Number of each fungus colonies}}{\text{Total number of fungi colonies}} \times 100$$

H.I. = Soil subjected to human impact.

J.A. = Soil surrounding the roots of a juvenile *Acacia saligna*.

mycolflora due to the human impact, the prevalent fungus (*Fusarium solani*) inside the protected area also recorded the highest frequency in the cultivated area, being 35%. *Alternaria alternata* (Fr.) Keissl., however, came second with relatively high frequency of 15%. Worthy to mention that *Aspergillus clavatus* Desm. (not detected inside Al-Ahrash protectorate) was found with a frequency of 5%. Another six fungi, however, were identified and with frequency from 5 to 10%.

To elucidate the existence effect of *Acacia saligna* growing in the study area on soil mycoflora, a soil sample was collected from juvenile *Acacia saligna* soil surrounding the roots to be compared with the previously collected soil surrounding the root of mature plant.

It is evident from Table (1) that only five fungi were isolated from juvenile plant soil out of the 18 fungi associated with the mature tree. The frequency of these fungi ranged from 5 to 25%. The increase in the number of fungi associated with mature trees might be due to transmission of these fungi from the surrounding area through irresponsible behaviour of some local inhabitants such as grazing of sheep and goats on land of Al-Ahrash protectorate.

It is interesting to note that *Ulocladium* sp. was only isolated from the soil of the juvenile plant. It was found at a frequency of 10%.

It is therefore suggested that, conditions of wildlife prevailing at Al-Ahrash protectorate not only encourage existence of annual plant flora (El-Sahhar *et al*., 2009), but also the mycolflora, especially the soil borne fungi.

3.1.2. Mycorrhizal fungi

Mycorrhizal fungi known as vesicular – arbuscular mycorrhizal (VAM) fungi are soil-borne fungi that establish an obligatory mutualistic symbiosis with many plant species. This association provides the fungus with carbohydrates, such as glucose and sucrose. In return the fungus can access phosphorus sources to the plant, being especially beneficial for the plant partner in nutrient – poor soils (Harrison, 2005).

Soil analysis proved that Al-Ahrash protectorate is low in its content of nutrients, being 41.2 ppm N, 36 ppm P and 120 ppm K on the average (El-Sahhar *et al.*, 2009). The prevailing conditions in the soil of the study area are encouraging the development of mycorrhizal fungi. This motivated the interest to study the mycorrhizal fungi associated with *Acacia saligna* in this area.

Identification of purified VAM fungi spores proved that the VAM propagules extracted from soil of study area belong to the fungal genus *Glomus*.

According to Bisby *et al.* (2009) Annual Checklist, *Glomus* (some 80 species) belongs to Family: Glomeraceae, Order: Glomerales, Class: Glomeromycetes and Phylum: Glomeromycota.

Results in Table (2) present the number of spores of VAM fungi per 50 g of the soil surrounding roots of *Acacia saligna* at Al-Ahrash protectorate. The average number of spores was 1230 with a range between 400 and 2200. The wide range and variation of spore numbers in different quadrates might be due to irresponsible behavior of some local inhabitants leading to disturbance of the soil in the study area. This agrees with Jasper *et al*. (1989) who proved that disturbance of the dry soil containing hyphae of a VAM fungus severely reduced the infectivity of the hyphae.

In this concern, Krishna and Dart (1984) found that mycorrhizal inoculation in a sterile soil increased dry matter of pearl millet and phosphorus uptake at levels less than 20 kg/ha. At higher P levels the mycorhizal effect was decreased. This suggests that inoculation with efficient VAM fungi could be extremely useful in P deficient soils.

Moreover, Benbrahim and Ismaili (2002) studied the effect of a single inoculation with VAM fungi and dual inoculation infection with VAM fungi and *Rhizobium* to increase nutrient uptake and plant growth in the soil low in plant available N and P. Investigated treatments increased shoot and root weights of *Acacia saligna*, nodulation and N and P content of the plants.

Table (2): Spore number of the mycorrhizal fungus *Glomus* **sp. in the soil surrounding the roots of** *Acacia saligna* **at Al-Ahrash protectorate.**

Quadrates No.	Spore No. / 50 g soil
1	2200
2	1300
3	700
4	2100
5	400
6	200
7	500
8	1200
9	1800
10	1900
Average	1230
Range	400 – 2200
H.I.	900
J.A.	7100

H.I. = Soil subjected to human impact.
J.A. = Soil surrounding the roots of a juvenile *Acacia saligna*.

Referring to the soil subjected to human impact in the area near to Al-Ahrash protectorate, the number recorded for spores of VAM fungi decreased to 900 spores indicating that the prevailing conditions in the study area were more suitable to VAM fungi compared with the area subjected to human impact.

On the contrary, the number of spores of VAM fungi increased to 7100 in the soil surrounding roots of the juvenile *Acacia saligna* compared with 1230 spores for mature plants. This increment could be attributed to the expected higher activity of all phenomena in the root of juvenile plant and the delicate primary structure of the root system which might facilitate penetration and propagation of VAM fungi through root cells.

It is welcomed to find VAM fungi in Al-Ahrash protectorate to facilitate the transfer of nutrient content, especially phosphorous in soil of the study area. This would be of great benefit to the natural vegetation occurred in such area.

3.2. Mycoflora of *Acacia saligna* phyllodes

Disease symptoms, mainly leaf spots were observed on phyllodes of *Acacia saligna*. Samples were collected from quadrates 2, 6, 9 and 10, where disease symptoms were detected (Table 3). It was possible to isolate seven fungi from diseased phyllodes. Three fungi of them had been previously isolated from the soil (Table 1); namely, *Alternaria alternata*, *Cladosporium herbarum* and *Stemphylium botryosum*.

Table (3): Frequency (%) of fungi associated with diseased *Acacia saligna* **phyllodes at Al-Ahrash protectorate.**

Fungi	Frequency (%) in quadrates				Average	Range
	2	6	9	10		
Alternaria alternata (Fr.) Keissel	30		30	20	20.0	20–30
Botryodiplodia theobromae Pat.	20	20	30	20	22.5	20-30
Cercospora sp.		10	20		7.5	10-20
Cladosporium herbarum (Pers.) Link	30	30		30	22.5	30-30
Colletotrichum sp.		20			5.0	0-20
Diplodia sp.		20	20		10.0	20-20
Stemphylium botryosum Sacc.	20			30	12.5	20-30

$$\text{Frequency}\,(\%) = \frac{\text{Number of each fungus colonies}}{\text{Total number of fungi colonies}} \times 100$$

Regarding the average frequency of isolated fungi, both *Botryodiplodia*

theobromae and *Cladosporium herbarum* recorded an average of 22.5% (range 20-30%), being the highest in this respect, followed by *Alternaria alternata* (20%). The remainder four fungi showed an average frequency between 5 and 12.5%, and a range from 0 to 30%.

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الفطريات المصاحبة لنبات الاكاشيا ساليجنا *Acacia saligna* **(Labill.) Wendl.**
النوع النباتي السائد في محميه الاحراش برفح – شمال سيناء - مصر

قاسم فؤاد السحار- عبير محمد شلتوت* – لما اسعد قاسم**

قسم النبات الزراعي – كلية الزراعة – جامعة القاهرة– الجيزة- مصر
*معهد بحوث امراض النباتات- مركز البحوث الزراعية – الجيزة- مصر
** قسم البيئة والحراج – كلية الزراعة – جامعة دمشق – الجمهورية العربية السورية

ملخص

تتناول هذه الدراسة الفطريات الموجودة بمحمية الاحراش التي تشغل مساحة 8 كم ^{2} وتبعد بمسافة 370 كم شرقي القاهرة ، حيث تقع في مدينة رفح المصرية ، محافظة شمال سيناء (18 / 31° شمالا 14 / 34° شرقا) والتي اعلنت محمية تنمية موارد طبقا لقراري رئيس مجلس الوزراء رقم 1429/ 1985 و 3379 / 1996 .

اشتملت الدراسة علي حصر وتعريف الفطريات المصاحبة لنبات الاكاشيا ساليجنا *Acacia saligna* (Labill.) Wendl النوع النباتي السائد في محمية الاحراش سواء بالتربة المحيطة بالجذور او تلك التي تصيب المجموع الخضري للنبات.

اتضح من حصر الفطريات المصاحبة للتربة المحيطة بجذور نباتات *Acacia saligna* ان الفطر *Fusarium solani* يحتل المرتبة الاولي في الانتشار بمتوسط 26.7% ونسبة تتراوح ما بين 10 – 45% وقد امكن عزل 20 نوعا من الفطريات بالتربة في المنطقة محل الدراسة، تسعة من هذه الانواع فطريات كامنه بالتربة والاحد عشر الاخري قد تصيب الاوراق النباتية . وقد سجل الفطر *Fusarium solani* اعلي قدرمن الانتشار،35% بالحقول المزروعة المجاورة. ارتفع عدد انواع الفطريات المصاحبة للنباتات البالغة من *Acacia saligna* مقارنة بالنباتات حديثة النمو .

أوضح تعريف الجراثيم النقية لفطريات الميكوريزا المستخلصة من تربة المنطقة محل الدراسة انتمائها لجنس *Glomus* وكان متوسط عدد الجراثيم في 50 جم تربة 1230 جرثومة، وتراوح فيما بين 400 – 2200، وقد انخفض هذا العدد الي 900 جرثومة في تربة الحقول المزروعة والمتاخمة لمحمية الاحراش . وعلي العكس من ذلك فقد ارتفع العدد الي 7100 جرثومة بالتربة المحيطة بجذور النباتات حديثة النمو.

امكن عزل سبعة انواع فطرية من نباتات *Acacia saligna* علي الاعناق المتورقة المصابة، حيث سجل كل من *Botryodiplodia theobromae* و *Cladosporium herbarum* متوسط تكرار قدره 22.5% وتراوح فيما بين 20 إلي 30 % وهما الاكثر من بقية الفطريات المعزولة.

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (60) العدد الثالث (يوليو 2009):342-350.