

Isolation and Identification of Endophytic Fungi from Leaves and Roots of *Althea rosea*

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Abstract: The present investigation was designed for studying the endophytic fungi of *Althea rosea* in Qena, potentialities of plant extracts (hydrophilic and hydrophobic) against dermatophytic fungi and pathogenic bacteria. The results obtained during this investigation could be summarized as follows:

Forty four species belonging to 27 genera were isolated and identified from young and old leaves and roots of *Althea rosea*, in addition to 3 types of sterile mycelium and unidentified fungi on glucose-Czapek's agar at 28±2° C. It can be seen from these results obtained, the number of isolates from old leaves and roots were more than the number of isolates from young leaves and roots also the number of isolates from midrib was more than from other parts.

I. INTRODUCTION

Endophytic fungi include all organisms that grow inside plant tissues without causing any immediate, negative effects and are not mycorrhizal (Saikkonen *et al.*, 2004; Macías-Rubalcava *et al.*, 2008; Souza *et al.*, 2008).

Fungal endophytes can be divided into two groups:-

1- The balansiaceous group: this group specifically colonizes grasses and usually belongs to the Ascomycetous genera *Epicholä* and *Balansia* (anamorphs *Neotyphodium* and *Ephelis*).

2- The non-balansiaceous group: this group occurs in almost all plant species and usually belongs to the Ascomycota (Wang *et al.*, 2007). Members of the Ascomycotina, Basidiomycotina, Deutromycotina, and Oomycetes have been isolated as endophytes (Petrini, 1986; Siegal *et al.*, 1987; Clay, 1991b). But, most fungal endophytes belong to Ascomycetes and fungi imperfecti (Valachova *et al.*, 2005). Some species of endophytic fungi have been identified as sources of anticancer, antidiabetic, insecticidal and immunosuppressive compounds (Strobel & Daisy, 2003; Ezra *et al.*, 2004). There are many reports demonstrating that many bioactive compounds could be produced by endophytic microorganisms (Huang *et al.*, 2001). Strains of the endophytic *Pezicula* species (and Its anamorph *Cryptosporiopsis*) from several deciduous and coniferous tree hosts produce an ensemble of bioactive secondary metabolites in culture (Fisher *et al.*, 1984; Nobel *et al.*, 1991; Schulz *et al.*, 1995). Several reviews have discussed the products of endophytic microbes and their promise for use in medicine, agriculture and industry (Tan & Zou, 2001; Strobel, 2002; Strobel & Daisy, 2003).

These endophytic microorganisms are ubiquitous and many increase the plant fitness by improving tolerance to heavy metals and drought, reducing the herbivory or phytopathogen settling (Waller *et al.*, 2008). The endophytic microorganisms are not considered as saprophytes since they are associated with living tissues and may in some way contribute to the well being of the plant. That is, the plant is thought to provide nutrients to the microbe, while the microbe may produce factors that protect the host plant from attack by animals, insects or microbes (Yang *et al.*, 1994;

Taechowisan *et al.*, 2005). There are many reports demonstrating that many bioactive compounds could be produced by endophytic microorganisms (Huang *et al.*, 2001; Christensen *et al.*, 2008).

II. MATERIALS & METHODS

Althea rosea was collected from different locations in South Valley University (Qena). Ten samples comprised some organs (leaves and roots) and different ages (old and young). Each sample was put in a sterile polyethylene bag, sealed and kept in another bag which was also sealed. Prolonged transport in sealed plastic bags, perforated bags designed for vegetable storage work well for transport and temporary storage of most types of plant tissues (Bills, 1996). Samples were transported on the same day to laboratory and were kept at (5°C) for mycological analysis.

Medium Used for Isolation of Plant-Borne Endophytic Fungi:

Non-selective and routine mycological medium is suitable for primary isolation of endophytic fungi and for subculturing. Glucose-Czapek's agar medium (glucose, 10g; NaNO₃, 2g; KH₂PO₄, 1g; MgSO₄·7H₂O, 0.5g; KCl, 0.5g; FeSO₄·7H₂O, 0.01g and agar, 15-20g per liter of distilled water) was used for isolation of endophytic fungi. Rose bengal (1/30000) and chloramphenicol (1/15000) were used as bacteriostatic and bacteriocidal agents (Smith & Dawson, 1944; Al-Doory, 1980; Redlin & Carris, 1996).

Determination of Mycoflora:

Surface Sterilization

Probably no other step is as critical to obtain good results as thorough, but non-penetrating surface sterilization. The possibility that isolates have been initiated from propagules on the surface must be minimized. The choice of sterilization times, concentration and volumes will be dictated by the thickness of sample, the relative permeability of its surface, and the texture of its surface (Redlin & Carris, 1996).

a) From leaves & roots

- 1) Ten healthy leaves or roots (5 old and 5 young) from each sample were used.
- 2) Serial washing in running tap water often was carried out to remove surface contamination in cases where a non-toxic method is desired.
- 3) Three 1cm diameter discs were cut from each leaf, from distal, central and proximal parts of the blade, but one 1 cm diameter discs were cut from each root, surface sterilized by sequential immersion in 75% ethanol for 1min (act as a surfactant), 0.93-1.3 M solution of sodium hypochlorite (3%-5% available chlorine) for 3 min (actual sterilizing agent) and 75% ethanol for 0.5 min, and rinsed in sterilized distilled water, then they were thoroughly dried between sterilized filter paper (Filip *et al.*, 2003).
- 4) These parts from each leaf and root were inserted in sterilized petri dishes containing glucose-Czapek's agar medium. The plates (5 plates) were incubated at 28±2°C for 2-3 weeks and the developing fungi were counted and identified.

III. RESULTS & DISCUSSION

The choice of glucose-Czapek's agar medium as isolation and identification medium because glucose-Czapek's agar medium supported good growth of all the common fungi, which produce well-formed colonies with good conidial production. Redlin & Carris (1996) showed that the majority of mycofloristic studies of complex organic substrates are based on methods using so-called "non-selective" media, which are in reality highly selective.

Forty species belonging to 24 genera were isolated and identified from young and old leaves comprising three parts of leaves (blade, midrib and petiole) in addition to young and old roots (Tables, 1, 2 and 3).

The gross fungal counts of *Althea rosea* plant from different organs at different ages were 135 colonies from young leaves, 284 colonies from old leaves, 183 colonies from young roots and 311 colonies from old roots.

It can be seen from these results that the number of isolates from old leaves and roots were more than the number of isolates from young leaves and roots (Tables, 2 & 3). Many investigations showed that foliar and stems endophytes are

that overall infection frequencies increase with the age of host organs or tissues (Fisher *et al.*, 1986; Stone, 1987; Rodrigues and Samules, 1990; Rodrigues, 1994). Also the numbers of fungal colonies isolated from midrib (52 and 114 isolates from young and old leaves, respectively) were higher than the other parts, shown in Table (1). Cannon and Simmons (2002) reported that the greater colonization of the midrib compared with that of laminar tissues presumably at least partially reflects the slightly larger size of the leaf fragments, but perhaps also the more complex anatomical structure.

Mycoflora of Altheae rosea Leaves

Table (2) showed that the number of total fungi isolated from young and old leaves equal 419 isolates (135 and 284 isolates from young and old leaves, respectively) represented by 34 fungal species of 21 genera.

Fusarium was the most dominant genus identified during this experiment. It was recovered from 8/10 and 5/10 samples, comprised 28.15% and 11.27% of total fungi of young and old leaves, respectively. *Fusarium* represented by 5 species (*F. anthophilum*, *F. dimerum*, *F. moniliforme*, *F. oxysporum* and *F. sambucinum*). Fusaria were extremely dominant in different plants such as *Fucus* sp., *Gossypium* sp., *Hypoxylon croceum*, *Manilkara bidentata*, *Mangrove* sp., *Taxus* sp. and *Theobroma cacao* L. (Ananda & Sridhar, 2002; Schulz *et al.*, 2002; Rubini *et al.*, 2005; Wang *et al.*, 2007). Wang *et al.* (2007) recovered *Fusarium* from *Gossypium* as endophytic fungi which occupied the third place in the count and comprised 19% of total fungi. Blodgett *et al.* (2007) isolated *Fusarium* species from roots of *Amaranthus hybridus* in South Africa which comprised 18% of total fungi. This genus was represented by 9 species of which *F. sambucinum* and *F. moniliforme* were the most predominant species. *F. moniliforme* was the most dominant species based on the number of cases of isolation (70% & 50% of the total samples tested from young and old leaves, respectively), and total counts (84.21% & 59.38% of total fusaria and 23.70% & 6.69% of total fungi, respectively). *F. moniliforme* was widely distributed in cacao branch plants as endophytic fungi (Rubini *et al.*, 2005). While, *F. sambucinum* and *F. anthophilum* were isolated with moderate frequencies of occurrence. They recovered from 1 & 3 and 2 & 1 samples, represented 2.63% & 25% and 5.26% & 12.5% of total fusaria and 0.741% & 2.817% and 1.481% & 1.408% of total fungi from young and old leaves, respectively. Loos *et al.* (2004) isolated *F. sambucinum* in 10% of total *Fusarium* species isolated during their studies about occurrence and distribution of *Microdochium nivale* and *Fusarium* species isolated from barely, durum and soft wheat grains in France.

The remaining *Fusarium* species (*F. dimerum* and *F. oxysporum*) were isolated from one sample. *F. dimerum* isolated from one sample of young leaves comprised 7.89% of total fusaria and 2.22% of total fungi, while *F. oxysporum* isolated from one sample of old leaves comprised 3.125% of total fusaria and 0.35% of total fungi (Table, 2). *F. oxysporum* was isolated from roots of mangrove plants and also from branch of *Theobroma cacao* Ananda & Sridhar (2002), Rubini *et al.* (2005). Shebany (2005) isolated all of these species in her studies on wheat plants.

Chaetomium was the second most common genus in count which comprised 2.22% and 15.14% of total fungi, from young and old leaves, respectively. *C. atrobrunneum* and *C. hexagonosporum* were isolated from the two ages of leaves, which collectively comprised 100% and 23.26% of total *Chaetomium* and 2.22% and 3.52% of total fungi from young and old leaves, respectively. The other *Chaetomium* species (*C. dreyfussii*, *C. globosum* and *C. variostiolatum*) were only detected in old leaves of *Altheae rosea* which represented 16.28%, 58.14% and 2.33% of total *Chaetomium* and 2.465%, 8.803% and 0.352% of total fungi, respectively. The genus was prevalent on leaves of *Manilkara bidentata* (Lodge *et al.*, 1996), from Sonoran desert plant (Wijeratne *et al.*, 2006) and from surface-disinfested soybean seeds (Sinclair & Backman, 1989). *Chaetomium* species were isolated as endophytic fungi from wheat leaves reduced the number and development of pustules of stem rust *Puccinia recondita* f. sp. *tritici* in wheat (Dingle & McGee, 2003; Istifadah *et al.*, 2006).

Eurotium was ranked the third place based on the count but occupied the first place with *Fusarium* according to the number of cases of isolation which isolated from 80% and 50% of the samples from young and old leaves, respectively, and comprised 13.33% and 9.15% of total fungi from young and old leaves, respectively.

Aspergillus was isolated from 4 samples in each age which constituted 8.89% & 7.39% of total fungi from young and old leaves, respectively. Of the genus, 5 species were collected of which *A. ochraceous* (41.67% and 28.57% of total *Aspergillus* and 3.7% and 2.11% of total fungi), and *A. niger* (33.3% and 19.05% of total *Aspergillus* and 2.96% and 1.41% of total fungi from young and old leaves, respectively). *A. terreus* and *A. versicolor* were isolated from the

two ages which collectively comprised 25% and 38.09% of total *Aspergillus* and 2.22% and 2.82% of total fungi from young and old leaves, respectively. *A. ustus* was the only *Aspergillus* which isolated from old leaves comprised 14.29% of total *Aspergillus* and 1.06% of total fungi. Aspergilli were extremely dominant in Egyptian medicinal plants (Abdel-Mallek *et al.*, 1990; Zohari *et al.*, 1992; El-Kady *et al.*, 1993; Youssef, 1995). Caruso *et al.* (2000) recovered *Aspergillus* sp. and *Cladosporium* sp. from different trees of genus *Taxus*, and isolated *Aspergillus* sp. from woody and herbaceous tissues but, *Cladosporium* sp. isolated only from herbaceous tissues of this genus.

Cladosporium (2 species) and *Penicillium* (4 species) were recovered in moderate frequencies from young and old leaves, which occurred in 4% and 3% of total samples and comprised 7.41% and 6.67% of total fungi from young leaves, respectively. But in old leaves, they were recovered from 6% and 3% of total samples and comprised 6.69% and 3.17% of total fungi, respectively (Table, 2). These species were recovered as endophytic fungi from many plants (Marlida *et al.*, 2000; Cannon & Simmons, 2002; Rubini *et al.*, 2005; Ganley & Newcombe, 2006; Weber *et al.*, 2007). *Cladosporium* sp. was collected from 30 trees samples throughout the trees limited range in Northern Florida (Lee *et al.*, 1995).

Acremonium sp., *Domingella asterinarum*, *Emericella nidulans*, *Myrothecium roridum* and *Paecilomyces variotii* were recovered from the plant in one age. *Paecilomyces variotii* and *Domingella asterinarum* were isolated from young leaves and other species isolated from old leaves, comprised 6.69%, 1.48%, 0.704%, 2.817% and 0.74% of total fungi, respectively. The remaining fungi were detected and identified from the two ages (young and old leaves) where the number of occurrence ranged between 1-3 and 1-5 samples in young and old leaves, respectively (Table, 2). The above mentioned species were previously isolated by several researchers from different plants (Fisher *et al.*, 1995; Strobel *et al.*, 1997; Daferner *et al.*, 1999; Caruso *et al.*, 2000; Ragazzi *et al.*, 2001; Ananda & Sridhar, 2002; Kumar *et al.*, 2004; Weber *et al.*, 2007). *Acremonium* was isolated from *Tripterium wilfordii* as endophytic fungi and studied as anti-proliferative activity on human peripheral blood mononuclear cells (Kumar *et al.*, 2004).

Mycoflora of Altheae rosea Roots

The number of total fungi isolated from young and old roots equal 494 isolates (183 and 311 isolates from young and old roots, respectively), and represented by 23 species and 1 variety of 16 genera (Table, 3).

Fusarium contributed the broadest spectrum of the total count in young and old roots (18.03% & 18.97% of total fungal count, respectively), while occupied the second place in number of cases of isolation 5/10 and 8/10 samples from young and old roots, respectively. Of the genus 5 species were identified of which *F. moniliforme* and *F. sambucinum* occurred in the two ages (young and old roots), *F. moniliforme* and *F. sambucinum* have the highest counts (collectively 100% & 89.83% of total fusaria and 18.03% & 17.04% of total fungi, from young and old roots, respectively). The remaining *Fusarium* species (*F. merismoides*, *F. oxysporum* and *F. scripi*) were detected in old roots only and disappeared in young roots. They represented by 3.39%, 5.08% and 1.69% of total fusaria and 0.643%, 0.965% and 0.322% of total fungi, respectively.

Chaetomium occupied the second place based on the fungal counts (15.3% & 11.25% of total fungi from young and old roots, respectively). But, ranked the first place in the number of cases of isolation which occurred in 60% and 100% of samples tested, respectively. Three species namely, *C. atrobrunneum*, *C. barilochense* and *C. globosum* were isolated and identified. Arrangement of these species according to count differed in young from old roots. In young roots, *C. barilochense* contributed the broadest spectrum in counts, followed by *C. globosum* then *C. atrobrunneum* (40%, 20% & 20% of the samples, 60.71%, 21.43% & 17.86% of total *Chaetomium* and 9.29%, 3.28% & 2.73% of total fungi, respectively), while in old roots, *C. globosum* was superior in count then *C. atrobrunneum* and the later was *C. barilochense* (60%, 30% & 50% of the samples, 40%, 31.43% & 28.57% of total *Chaetomium* and 4.5%, 3.54% & 3.22% of total fungi, respectively).

Aspergillus occupied the third place according to the count comprised 8.74% and 8.68% of total fungi from young and old roots, respectively. Of the genus 5 species were identified. *A. flavus* and *A. terreus* were a head based on total fungal counts (31.25% & 33.3% of total *Aspergillus* and 2.73% and 2.89% of total fungi from young and old roots, respectively). Each species was recovered from 20% of total samples from each age. *A. niger* followed these species in the count represented 18.75% and 25.93% of total *Aspergillus* and 1.64% and 2.25% of total fungi from young and old roots, respectively, and isolated from 20% of total samples from young roots and 30% of the samples from old roots. The

remaining species isolated only from one age, where *A. ochraceus* isolated from 10% of total samples from old roots comprising 7.41% of total *Aspergillus* and 0.64% of total fungi, while *A. versicolor* isolated from one sample of young roots comprising 18.75% of total *Aspergillus* and 1.64% of total fungi.

Cirrenalia sp. and sterile mycelium (white yellow) were recovered with moderate frequencies, each observed from 50% of total samples collectively contributed 26.78% and 20.58% of total fungi from young and old roots, respectively.

Acremonium sp., *Cladosporium* (2 species), *Eurotium repens*, *Myrothecium roridum*, *Phoma* sp., *Torula herbarum*, *Verticillium lateritium*, sterile mycelium (black) and unidentified fungal species were isolated in the two ages with moderate or low counts, comprised collectively 28.4% and 28.3 % of total fungi isolated from *Althea rosea* young and old roots, respectively (Table, 3). The remaining fungal species occurred in one age. Most species were isolated from old roots with low frequencies; comprised 7.075 % of total fungi whereas, *Saccharomyces* sp. was isolated from young roots comprised 2.19 % of total fungi (Table, 3).

Table (1): Total counts of fungal genera and species isolated from blade, midrib and petiole (each, 100 segments) of *Althea rosea* leaves (young and old) on glucose-Czapek's agar at 28±2 °C.

Genera and species	Young leaves			Old leaves		
	Blade	Midrib	Petiole	Blade	Midrib	Petiole
<i>Acremonium</i> sp.	0	0	0	3	8	8
<i>Alternaria alternata</i>	1	3	0	3	1	1
<i>Aphanocladium album</i>	1	1	1	4	0	4
<i>Aspergillus</i>	3	7	2	9	6	6
<i>A. niger</i>	1	2	1	2	1	1
<i>A. ochraceus</i>	2	3	0	3	2	1
<i>A. terreus</i>	0	1	0	2	2	1
<i>A. ustus</i>	0	1	0	1	1	1
<i>A.versicolor</i>	0	1	1	1	0	2
<i>Chaetomium</i>	1	2	0	8	24	11
<i>C. atrobrunneum</i>	1	1	0	0	4	1
<i>C. dreyfussii</i>	0	0	0	1	4	2
<i>C. globosum</i>	0	0	0	5	3	1
<i>C. hexagonosporum</i>	0	1	0	1	13	7
<i>C. variostiolatum</i>	0	0	0	1	0	0
<i>Cirrenalia</i> sp.	1	2	1	4	5	3
<i>Cladasporium</i>	5	3	2	4	6	9
<i>C. cladosporioides</i>	5	3	2	3	1	3
<i>C. sphaerospermum</i>	0	0	0	1	5	6
<i>Domingella asterinarum</i>	1	0	1	0	0	0
<i>Drechslera halodes</i>	1	1	1	0	2	0
<i>Emericella nidulans</i>	0	0	0	1	0	1
<i>Eurotium repens</i>	4	6	8	6	11	9
<i>Fusarium</i>	14	13	11	13	7	12
<i>F. anthophilum</i>	1	1	0	3	1	0
<i>F. dimerum</i>	1	1	1	0	0	0
<i>F. moniliforme</i>	12	10	10	8	3	8
<i>F. oxysporum</i>	0	0	0	0	1	0
<i>F. sambucinum</i>	0	1	0	2	2	4
<i>Microascus trigonosporous</i>	0	1	0	4	6	4
<i>Monilia fructigena</i>	1	1	0	2	2	2
<i>Myrothecium roridum</i>	0	0	0	1	6	1

<i>Nigrospora oryzae</i>	2	1	2	2	6	4
<i>Penicillium</i>	1	6	2	2	2	5
<i>P. chrysogenum</i>	0	1	0	0	0	2
<i>P. corylophilum</i>	0	2	0	0	0	0
<i>P. jensenii</i>	1	2	1	0	0	0
<i>P. verruculosum</i>	0	1	1	2	2	3
<i>Paecilomyces variotii</i>	1	0	0	0	0	0
<i>Phoma</i> sp.	1	2	1	5	8	4
<i>Torula herbarum</i>	1	0	2	0	0	3
<i>Ulocladium botrytis</i>	1	0	1	3	2	2
Sterile mycelium (black)	1	0	1	1	3	1
Sterile mycelium (white yellow)	1	2	1	2	8	1
Sterile mycelium (yellow)	0	0	2	1	1	0
Unidentified fungi	1	1	1	1	0	0
Gross total count	43	52	40	79	114	91
No. of genera	17	14	13	17	16	18
No. of species	18	23	14	24	23	25

Table(2): Total counts (calculated per 150 young or old leaf segments), percentage counts(% C, calculated per total fungi), percentage frequency (%F, calculated per 10 samples) and number of cases of isolation (NCI, out of 10 samples) of fungal genera and species recovered from leaves of *Althea rosea* on glucose-Czapek's agar at 28 ±2° C.

Genera and species	Young leaves				Old leaves			
	TC	C%	NCI	F%	TC	C%	NCI	F%
<i>Acremonium</i> sp.	0	0.000	0	0	19	6.690	4	40
<i>Alternaria alternata</i>	4	2.963	3	30	5	1.761	2	20
<i>Aphanocladium album</i>	3	2.222	1	10	8	2.817	2	20
<i>Aspergillus</i>	12	8.89	4	40	21	7.39	4	40
<i>A. niger</i>	4	2.963	1	10	4	1.408	1	10
<i>A. ochraceus</i>	5	3.704	1	10	6	2.113	1	10
<i>A. terreus</i>	1	0.741	1	10	5	1.761	1	10
<i>A. ustus</i>	1	0.741	1	10	3	1.056	1	10
<i>A.versicolor</i>	2	1.481	1	10	3	1.056	2	20
<i>Chaetomium</i>	3	2.222	2	20	43	15.142	7	70
<i>C. atrobrunneum</i>	2	1.481	2	20	5	1.761	3	30
<i>C. dreyfussii</i>	0	0.000	0	0	7	2.465	4	40
<i>C. globosum</i>	0	0.000	0	0	25	8.803	6	60
<i>C. hexagonosporum</i>	1	0.741	1	10	5	1.761	3	30
<i>C. variostiolatum</i>	0	0.000	0	0	1	0.352	1	10
<i>Cirrenalia</i> sp.	4	2.963	2	20	12	4.225	5	50
<i>Cladasporium</i>	10	7.407	4	40	19	6.690	6	60
<i>C. cladosporioides</i>	10	7.407	4	40	7	2.465	3	30
<i>C. sphaerospermum</i>	0	0.000	0	0	12	4.225	4	40
<i>Domingella asterinarum</i>	2	1.481	1	10	0	0.000	0	0
<i>Drechslera halodes</i>	3	2.222	1	10	2	0.704	1	10
<i>Emericella nidulans</i>	0	0.000	0	0	2	0.704	1	10
<i>Eurotium repens</i>	18	13.330	8	80	26	9.150	5	50
<i>Fusarium</i>	38	28.150	8	80	32	11.267	5	50
<i>F. anthophilum</i>	2	1.481	2	20	4	1.408	1	10

<i>F. dimerum</i>	3	2.222	1	10	0	0.000	0	0
<i>F. moniliforme</i>	32	23.704	7	70	19	6.690	5	50
<i>F. oxysporum</i>	0	0.000	0	0	1	0.352	1	10
<i>F. sambucinum</i>	1	0.741	1	10	8	2.817	3	30
<i>Microascus trigonosporous</i>	1	0.741	1	10	14	4.929	4	40
<i>Monilia fructigena</i>	2	1.481	1	10	6	2.113	1	10
<i>Myrothecium roridum</i>	0	0.000	0	0	8	2.817	4	40
<i>Nigrospora oryzae</i>	5	3.704	2	20	12	4.225	4	40
<i>Penicillium</i>	9	6.666	3	30	9	3.170	3	30
<i>P. chrysogenum</i>	1	0.741	1	10	2	0.704	2	20
<i>P. corylophilum</i>	2	1.481	2	20	0	0.000	0	0
<i>P. jensenii</i>	4	2.963	1	10	0	0.000	0	0
<i>P. verruculosum</i>	2	1.481	2	20	7	2.465	1	10
<i>Paecilomyces variotii</i>	1	0.741	1	10	0	0.000	0	0
<i>Phoma</i> sp.	4	2.963	1	10	17	5.986	5	50
<i>Torula herbarum</i>	3	2.222	2	20	3	1.056	1	10
<i>Ulocladium botrytis</i>	2	1.481	1	10	7	2.465	3	30
Sterile mycelium (black)	2	1.481	2	20	5	1.761	2	20
Sterile mycelium (white yellow)	4	2.963	2	20	11	3.873	5	50
Sterile mycelium (yellow)	2	1.481	1	10	2	0.704	2	20
Unidentified fungi	3	2.222	1	10	1	0.352	1	10
Gross total count	135				284			
No. of genera	18				19			
No. of species	26				29			

Table (3): Total counts (calculated per 200 young or old root segments), percentage counts(% C, calculated per total fungi), percentage frequency (%F, calculated per 10 samples) and number of cases of isolation (NCI, out of 10 samples) of fungal genera and species recovered from roots of *Althea rosea* on glucose-Czapek's agar at 28 ± 2 °C.

Genera and species	Young roots				Old roots			
	TC	C%	NCI	F%	TC	C%	NCI	F%
<i>Acremonium</i> sp.	6	3.280	2	20	11	3.537	3	30
<i>Aspergillus</i>	16	8.74	3	30	27	8.68	4	40
<i>A. flavus</i>	5	2.730	2	20	9	2.894	2	20
<i>A. niger</i>	3	1.640	2	20	7	2.251	3	30
<i>A. ochraceus</i>	0	0.000	0	0	2	0.643	1	10
<i>A. terreus</i>	5	2.730	2	20	9	2.894	2	20
<i>A. versicolor</i>	3	1.640	1	10	0	0.000	0	0
<i>Chaetomium</i>	28	15.300	6	60	35	11.254	10	100
<i>C. atrobrunneum</i>	5	2.730	2	20	11	3.537	3	30
<i>C. barilochense</i>	17	9.290	4	40	10	3.215	5	50
<i>C. globosum</i>	6	3.280	2	20	14	4.502	6	60
<i>Cirrenalia</i> sp.	16	8.740	4	40	35	11.254	6	60
<i>Cladasporium</i>	13	7.100	2	20	4	1.286	1	10
<i>C. cladosporioides</i>	9	4.920	2	20	4	1.286	1	10
<i>C. sphaerospermum</i>	4	2.190	1	10	0	0.000	0	0
<i>Emericella nidulans</i>	0	0.000	0	0	5	1.608	1	10
<i>Eurotium repens</i>	7	3.83	2	20	25	8.04	3	30

<i>Fusarium</i>	33	18.030	5	50	59	18.972	8	80
<i>F. merismoides</i>	0	0.000	0	0	2	0.643	1	10
<i>F. moniliforme</i>	25	13.660	4	40	31	9.968	5	50
<i>F. oxysporum</i>	0	0.000	0	0	3	0.965	2	20
<i>F. sambucinum</i>	8	4.370	2	20	22	7.074	6	60
<i>F. scripi</i>	0	0.000	0	0	1	0.322	1	10
<i>Myrothecium roridum</i>	4	2.190	2	20	11	3.537	6	60
<i>Penicillium verruculosum</i>	1	0.546	1	10	0	0.000	0	0
<i>Phoma</i> sp.	4	2.190	1	10	12	3.859	2	20
<i>Rhizopus stolonifer</i>	0	0.000	0	0	4	1.286	1	10
<i>Saccharomyces</i> sp.	4	2.190	1	10	0	0.000	0	0
<i>Torula herbarum</i>	7	3.830	3	30	2	0.643	1	10
<i>Ulocladium botrytis</i>	0	0.000	0	0	3	0.965	1	10
<i>Verticillium lateritium</i>	2	1.090	1	10	6	1.929	3	30
Sterile mycelium (black)	3	1.640	1	10	26	8.360	3	30
Sterile mycelium (white yellow)	33	18.030	3	30	29	9.322	7	70
Sterile mycelium (yellow)	0	0.000	0	0	5	1.608	3	30
Unidentified fungi	6	3.28	2	20	12	3.859	3	30
Gross total count	183	100			311	100		
No. of genera	13				14			
No. of species	16				20			

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