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DIVERSITY OF MYCOBIOTA ASSOCIATED WITH ONION (*ALLIUM CEPA* L.) CULTIVATED IN ASSIUT, WITH A NEWLY RECORDED FUNGAL SPECIES TO EGYPT

Khayria M. Abdel-Gawad[1], *Ahmed Y. Abdel-Mallek*[1, 2], *Nemmat A. Hussein*[1] *and Ismail R. Abdel-Rahim*[1]

Address(es):
[1]Botany & Microbiology Department, Faculty of Science, Assiut University, Egypt.
[2]Umm Al-Qura University, Faculty of Applied Science, Biology Department, Mecca, Kingdom of Saudi Arabia.

*Corresponding author: ismailramadan2008@gmail.com

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ABSTRACT

The goal of this study was to characterize diversity of fungal biota in soil, roots and green leaves of onion plant. Seventy- nine fungal species belonging to 32 genera were isolated from soil (29 genera and 72 species), rhizosphere (25 and 52), rhizoplane (24 and 38), phyllosphere (17 and 41) and phylloplane (17 and 35) on PDA medium at 19º and 28ºC. The number of fungal genera and species in soil was higher than those on roots and leaves, while those on the surface of roots (rhizosphere) or leaves (phyllosphere) were higher than those adhering to roots (rhizoplane) or leaves (phylloplane). *Aspergillus* (*A. niger* and *A. terreus*), followed by *Penicillium* (*P. funiculosum* and *P. chrysogenum*), *Rhizopus* (*R. stolonifer*) and *Fusarium* (*F. oxysporum*) were the most common fungi. A new record species is reported for the first time to Egypt namely, *Zopfiella latipes* (from phylloplane of onion).

Keywords: Soil, onion, *Allium cepa*, rhizosphere, rhizoplane, phyllosphere, phylloplane, *Zopfiella latipes*

INTRODUCTION

Onion (*Allium cepa* L., Alliaceae) is one of the main important and oldest vegetable crops grown in Egypt. Onion although primarily grown for food, is also used in traditional medicine, including the treatment of chicken pox, the common cold, influenza, measles and rheumatism. Antimicrobial characteristics of the *Allium* are related to the effect of sulfur compounds produced in its tissues. Onion may help to prevent arteriosclerosis and other cardiovascular diseases **(Schwartz and Mohan, 2007)**. The phyllosphere of plants is a dynamic ecosystem inhabited by specific bacteria and fungi. Their activity is related to various interactions between the biotic and abiotic factors of the environment **(Behrendt *et al.*, 1997, 2002)**. Saprotrophic leaf surface fungi perform key ecological roles in the plant and aerial plant surfaces provide a suitable habitat for epiphytic microorganisms, which are influenced by the nutrients present on the leaf surfaces **(Tyagi *et al.*, 1990; Abdel-Hafez *et al.*, 2015)**. Phylloplane provides a suitable habitat for the growth of microorganisms which can compete with the pathogen for nutrients and inhibit pathogen multiplication by secreting antibiotics or toxins **(Yadav *et al.*, 2011; Thakur and Harsh, 2014)**. Several studies were carried out to characterize the mycobiota of root surface and soil adhering the roots of onion plants. *Penicillium*, *Aspergillus*, *Trichoderma* and *Cladosporium* were detected from the rhizosphere of onion seedling **(Lyndsay, 1973).** In another study, 5 Zygomycetous species, 9 Ascomycetous species and 59 Hyphomycetous species were isolated from the rhizosphere of *Allium cepa* **(Bertoldi *et al.*, 1978)**. **Abdel-Sater (2001)** identified twenty fungal species from leaf surfaces of onion plant of which *Alternaria alternata, Aspergillus niger, A. sydowii, A. versicolor, Cladosporium herbarum, Cochliobolus lunatus, Pleospora herbarum, Setosphaeria rostrata* and *Ulocladium botrytis* were the most prevalent. **Montes-Belmont *et al.* (2003)** isolated also *Fusarium, Rhizoctonia, Curvularia, Phoma, Alternaria, Sclerotium, Bipolaris, Aspergillus, Rhizopus* and *Penicillium* from onion nurseries. *Fusarium culmorum, Penicillium* and *Colletotrichum circinans* were also reported as pathogens for onion bulbs and it is recommended to use eco-friendly root and leaf surface microorganisms to manage plant pathogens **(Abo-Shady *et al.*, 2007; Soria *et al.*, 2012; Abo-Elyousr *et al.*, 2014)**. Hence, it is necessary to determine the fungal populations in the soil, root and leaf regions which could have positive or negative impact on onion growth and development. This study aimed to provide comprehensive information on the fungi associated with soil, rhizosphere, rhizoplane, phyllosphere and phylloplane of onion (Giza 6) during the period from planting till harvesting.

MATERIAL AND METHODS

Collection of Samples

Two localities in Assiut Governorate were selected for the present study; Botanical Garden of Faculty of Science, Assiut University and Refa Village (12 Km south of Assiut city). Samples were collected monthly during the growing season which extended from September 2005 to April 2006.

Soil samples: Twenty-six soil samples were collected at a depth of 5 inches, put in sterilized polyethylene bags and mixed thoroughly and transferred directly to the laboratory **(Johnson *et al.*, 1959).**

Root samples: Onion roots (20 samples) were uprooted from the soil and shaken gently to collect the adhering soil. Then the roots and soil were placed separately in sterilized polyethylene bags and transferred to laboratory.

Samples of green leaves: For determination of phyllosphere and phylloplane fungi, 20 samples of green tubular leaves of onion were collected by cutting using sterilized scissors and packed directly into polyethylene bags and transferred to laboratory.

Isolation of Fungi

Soil borne fungi: Potato Dextrose Agar medium (PDA) supplemented with rose-bengal (0.067g/l) and chloramphenicol (0.25 g/l) as bacteriostatic agents **(Smith and Dawson, 1944; Booth, 1971)** was used. The dilution-plate method was employed to determine soil fungi **(Johnson *et al.*, 1959; Moubasher *et al.*, 1977)**. One ml of the desired dilution was transferred directly into each of sterilized 9 cm diameter Petri dishes, then, ~20ml of PDA were poured in each plate and stirred gently for homogenous distribution of soil suspension. The plates were incubated either at 19ºC and 28ºC for 7 days (five replicates for each sample). The developing colonies were enumerated and identified.

Rhizosphere fungi: The dilution plate method was used to isolate rhizosphere fungi. The PDA plates were incubated at either 19ºC or 28ºC (five replicates for each sample) for 7 days during which the developing fungi were counted and identified.

Rhizoplane fungi: The previously uprooted roots of onion plant were subjected for a series of washings with sterilized distilled water, dried, cut into equal segments (1 cm long). Five segments were placed on the surface of the PDA medium plates. The plates were incubated at 19ºC and 28ºC (5 replicates for each sample) for 7 days during which the developing colonies were counted and identified.

Phyllosphere fungi: Green leaves of onion were cut into segments (1 cm diam each). Twenty g of these segments were placed in sterile conical flasks containing 100 ml sterile distilled water and were shaken for 20 minutes. Final desired dilution (1/500) was prepared. One ml of the final dilution was transferred into sterilized Petri dish, and then 10-15 ml of melted PDA medium was poured and shaken gently. The plates were incubated at 19ºC and 28ºC for 7 days (5 replicates for each sample). Developing colonies were identified and counted.

Phylloplane fungi: The previous segments of onion green leaves were washed several times with sterilized distilled water. Then they were dried thoroughly between sterilized filter paper. Five segments (1 cm diam.) were placed on the surface of PDA plate. Five replicates were used for each sample and the plates were incubated at 19º and 28ºC. The developing fungi were counted and identified.

Identification of fungi

The fungal colonies were identified based on macro- and microscopic characters following **Raper and Fennell (1965)**, for *Aspergillus* species; **Ellis (1971, 1976)**, for Dematiaceous Hyphomycetes; **Booth (1971)**; **Leslie and Summerell (2006)**, for *Fusarium* species; **Pitt (1979)**, for *Penicillium* species; **Moubasher (1993)**, **Pitt and Hocking (1997)** and **Domsch *et al.* (2007)** for fungi in general.

Statistical analysis

Hierarchical clustering analysis using free online software statistical analysis (www.wessa.nit.com) was used and Detrended Correspondence Analysis (DCA) was performed using Canoco 4.5 **(Ter Braak and Šmilauer, 1998)** to ordinate sources based on their fungal composition.

RESULTS AND DISCUSSION

Collection of Samples

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RESULTS AND DISCUSSION

Seventy-nine species belonging to 32 genera were identified from soil (58 species and 25 genera), rhizosphere (47 and 23), rhizoplane (30 and 16), phyllosphere (37 and 15) and phylloplane (28 and 14) on PDA medium at 19°C. While lower number of genera (insert the number) and species (insert the number) were recovered at 28ºC from soil (26 genera, 60 species), rhizosphere (19 genera, 40 species), rhizoplane (13 genera, 28 species), phyllosphere (14 genera, 36 species) and from phylloplane (12 genera, 25 species) (Table 1).

Fungi isolated from soil samples

Seventy-two species appertaining to 29 genera were isolated from soil cultivated with onion plant on PDA plates incubated at 19° and 28°C. *Aspergillus*, *Penicillium*, *Cochliobolus*, *Fusarium* and *Rhizopus* were the most common genera at both 19° and 28°C. They were recorded in 65.38 - 100% of total samples tested (Table 1). The gross total fungal count was higher at 19°C than at 28°C as shown in table (1).

Aspergillus was represented by 9 and 8 species comprising 33.30 and 50.21% of total fungi at 19° and 28°C respectively. *A. niger* and *A. terreus* were isolated in high frequencies (ranging between 73.08% and 100% of total samples tested). On the other hand, *A. sydowii* was recorded only at 19°C with 11.54% frequency of occurrence, while two species (*A. carbonarius* and *A. oryzae*) were isolated only at 28°C. *A. niger* was reported as an abundant soil-borne fungus and may be a source of black mould of onion **(Tyson and Fullerton, 2004).**

Penicillium occurred in 84.62% and 88.46% of total samples at 19° and 28°C respectively. It was represented by 12 species of which, *P. funiculosum* was the most common, followed by *P. chrysogenum* at 19ºC and *P. oxalicum* at 28ºC. *P. islandicum* and *P. mirabile* were detected only at 19°C, but *P. pinophilum* was isolated at 28°C.

Cochliobolus (3 species), with its predominant species *C. spicifer*, was detected in 76.92% of total samples. *C. hawaiiensis* was isolated from 3.85% of total samples at only 28°C (Table 1).

Fusarium was represented by 6 and 5 species at 19° and 28°C respectively. *F. oxysporum* was the most common species, followed by *F. solani*. *F. equiseti*, *F. tricinctum* and *F. xylarioides* were isolated only at 19°C, and *F. oxysporum* var. *redolens* and *F. subglutinans* were isolated rarely at 28°C only.

Rhizopus stolonifer appeared in 84.62% and 65.38% of total samples, accounting 6.56% and 6.43% of total fungi at 19° and 28°C respectively (Table 1). The remaining genera (20 genera at 19°C and 21 genera at 28°C) were recorded in moderate or low frequency of occurrence (Table 1).

Several fungal genera and species were commonly isolated from soil in Egypt **(Abdel-Hafez *et al*., 2000; Zohri *et al*. 2014; Elkhateeb *et al*., 2016)**.

Rhizosphere fungi

Fourty-seven and 40 fungal species belonging to 23 and 19 genera constituting 679945 and 572980 cfu/g were isolated from 20 rhizosphere samples on PDA at 19° and 28°C respectively (Table 1). The number of species in the rhizosphere (52 species) was less than that in the soil away from it (72 species). This is probably due to that exudates secreted from onion roots preventing non-rhizosphere fungi to gain access to the rhizosphere. Our results are in harmony with those of **Sule and Oyeyiola (2012)**, while in contrast with those of **Mehrotra and Kakkar (1972)** who recorded greater number of fungi in the rhizosphere than in the soil. *Aspergillus* (8 species) comprised 54.49% and 63.32% of total fungi at 19° and 28°C respectively. *A. niger* was the predominant species followed by *A. terreus* and *A. versicolor* were isolated in moderate frequency at 28°C and in low frequency at 19°C. *A. flavipes* was isolated only at 28°C and *A. niveus* at 19°C only (Table 1). *Penicillium* was represented by 10 and 9 species matching 14.75 and 15.18% of total fungi at 19° and 28°C respectively. *P. funiculosum* was isolated moderately, while the remaining *Penicillium* species occurred in low frequency. The results showed that *P. chrysogenum*, *P. mirabile* and *P. pinophilum* were recorded at 19°C only, whereas, *P. citrinum* and *P. islandicum* were isolated at 28°C only. *Rhizopus stolonifer* occurred in 65% of the total samples tested, matching 6.26% and 6.75% of total fungi at 19° and 28°C respectively. Our results indicated that, *Acrophialophora fusispora*, *Botrytis cinerea*, *Humicola grisea*, *Rhizoctonia solani*, *Setosphaeria rostrata* and *Stemphylium botryosum* were isolated at 19°C only, while, *Cunninghamella echniulata* and *Macrophomina phaseolina* were isolated at 28°C only.

The above species were frequently recovered from rhizosphere and non rhizosphere soils of various plants cultivated in different localities of Egypt as reported by several workers **(Moubasher and Abdel-Hafez, 1986; Abdel-Hafez *et al.*, 1990, 2000; Elkhateeb *et al.*, 2016)**.

Rhizoplane fungi

Thirty and 28 species belonging to 16 and 13 genera were collected from onion roots at both 19° and 28ºC respectively on PDA medium (Table 1). The gross fungal count was slightly higher at 28°C (1327 cfu/25 root segments) than at 19°C (1276 cfu/25). It is worthy to mention that, the rhizosphere of onion roots hosted a broader spectrum of species than that of the rhizoplane (53 and 38 species respectively). This is in harmony with the results obtained by **Sule and Oyeyiola (2012)** who isolated 30 and 18 different fungal species as rhizosphere and rhizoplane fungi respectively. Also, **Porras-alfaro *et al.* (2011)** demonstrated that microbial richness in rhizosphere and soil samples was nearly three times greater than the richness described for fungal communities associated with roots of plants at the same site. *Aspergillus* was represented by 5 species, comprising 90% and 100% at 19° and 28°C respectively (Table 1). *A. niger* was the most prevalent species at 19°C and at 28°C. *A. sydowii* was isolated only at 19°C, while *A. ochraceus* was detected only at 28°C. The remaining species were recorded in low or rare frequencies. *Fusarium*, with its dominant species *F. oxysporum*, was represented by 2 and 3 species, comprising 19.98% and 12.51% of total fungi and occurred in 90% and 70% of the samples at 19° and 28°C respectively. *F. equiseti* was isolated rarely at 28°C only. *Fusarium oxysporum* and *F. solani* altogether with other fungal species were isolated from onion roots and bulbs in Northeast of Iran **(Rabiei- Motlagh *et al.*, 2010).** *Penicillium* (9 and 8 species) and *Rhizopus (R. stolonifer*) were recorded in high frequency at 19° and 28°C. *Botrytis cinerea, Chaetomium globosum, Cunninghamella echinulata, Emericella nidulans, Epicoccum nigrum, Phoma leveillei* and *Rhizoctonia solani* were isolated at 19°C only, but *Acremonium strictum, Macrophomina phaseolina, Setosphaeria rostrata* and *Sordaria fimicola* were isolated at 28°C only (Table 1). Several of these species were infrequently recovered from rhizoplane of some plants cultivated in Egypt **(Moubasher and Abdel-Hafez, 1986; Abdel-Hafez *et al.*, 1990, 2000)**.

Phyllosphere fungi

Fifteen genera and 37 species (at 19°C); 14 genera and 36 species (at 28°C) were recorded as phyllosphere fungi from green leaves of onion (Table 1). The total counts were slightly higher at 19°C (133500 cfu/g green leaves) than at 28°C (131300). *Aspergillus* (8 and 7 species, comprising 33.4% and 38.54% of total fungi at 19° and 28°C respectively), *Cladosporium* (3 species, 23.1% and 21.25% of total fungi), followed by *Penicillium* (10 and 9 species, 10.8% and 16.76% of total fungi) were the most common genera (Table 1). *Cladosporium* spp. are active at low temperature and high humidity and are known as important pathogens to plant leaves **(Kwon *et al.*, 2001)**. Other four fungal species were isolated at 19°C only (*A. oryzae*, *Gliocladium roseum, P. waksmanii* and *Stachybotrys chartarum*), while 3 species were detected at 28°C only (*C. hawaiiensis, F. verticillioides* and *Mucor circinelloides*).

Abdel-Hafez *et al.* (2015) isolated 58 fungal species belonging to 25 genera as phyllosphere fungi from healthy leaves of onion plant. *Alternaria alternata, Aspergillus niger, A. terreus, Cladosporium cladosporioides*, *Penicillium funiculosum,* and *Trichoderma harzianum* were recovered in high frequency, while *Aspergillus carbonarius, A. flavipes, Cunninghamella echinulata, Epicoccum nigrum, Humicola grisea, Myrothecium verrucaria, Nigrospora sphaerica*, *Penicillium citrinum* and other fungi were rarely isolated.

Phylloplane fungi

Fourteen and 12 genera including 28 and 25 species and contributing 811 and 944 cfu/25 leaf segments were isolated at 19° and 28°C respectively (Table 1). *Aspergillus* (7 species) was recorded in all samples matching 36.50% and 49.68% of total fungi at 19° and 28°C respectively. *A. niger* was the most common species, detected in all samples, contributing 28.24% and 40.89% of total fungi at 19° and 28°C respectively. *A. versicolor* was isolated at 19°C only, while *A. ustus* was recorded at 28°C only. The remaining species were infrequent (Table 1). *Penicillium* was isolated in moderate frequency (65% of samples) at 19°C representing 12.59% of total fungi and high frequency at 28°C (13.56% and 70% respectively). It was represented by 7 and 6 species at 19° and 28°C respectively. *P. funiculosum* was isolated moderately occurred in 40% and 55% of total samples, comprising 8.26% and 10.81% of total fungi at 19° and 28°C respectively. It is worthy to mention that, *P. corylophilum, P. fellutanum, P. mirabile* and *P. waksmanii* were recorded at 19°C only, while *P. citrinum, P. duclauxii* and *P. pinophilum* were isolated at 28°C only. The remaining *Penicillium* species (*P. chrysogenum* and *P. oxalicum*) were recovered in low frequency at both 19° and 28°C (Table 1). *Cladosporium* (2 species) and *Alternaria alternata* were isolated in moderate occurrence, while *Rhizopus stolonifer* was isolated in moderate and low frequencies at 28° and 19°C, respectively. In most reports, there is a marked dominance of anamorphic fungi, mostly of ascomycetous affinity, and the main genera found in the phylloplane are *Cladosporium*, *Aspergillus*, *Alternaria*, *Aureobasidium* and *Epicoccum* **(Pereira *et al.*, 2002; Guimarães *et al.*, 2011)**. Results in Table (1) revealed that, *Beauveria bassiana*, *Macrophomina phaseolina*, *Phoma leveillei* and *Rhizoctonia solani* were recorded at 19°C only, while *Acremonium strictum* and *Zopfiella latipes* were isolated at 28°C only in rare frequencies. *Acremonium strictum*, *Alternaria alternate*, *Aspergillus flavus*, *A. fumigates*, *A. niger*, *A. sydowii*, *A. terreus*, *Cladosporium cladosporioides*, *C. sphaerospermum*, *Fusarium oxysporum*, *Gliocladium roseum*, *Penicillium duclauxii*, *P. pinophilum*, *Stemphylium botryosum* and *S. vesicarium* were isolated previously from phylloplane of healthy green leaves of onion **(Abdel-Hafez *et al.*, 2015)**. *Aspergillus niger* and *Cladosporium cladosporioides* were also the most common species of leaf surface of onion as recorded previously by **Abdel- Sater (2001)**.

In the present study, the number of phyllosphere fungi of green leaves (41 species) exceeded that of phylloplane fungi (33 species). This means that about 22% of fungal species are not really inhabitants of the leaf surface, but are deposited from the air. These results are in agreement with those reported by **Abdel- Sater (2001)** and **Abdel-Hafez *et al.* (2015)**, who examined the leaf surface fungi of onion plants and reported that the number of phyllosphere fungi (58 and 20 species respectively) outnumbered those of the phylloplane (25 and 9 respectively). Results of the present study revealed that, the lowest number of genera (17 and 17) and species (41 and 35) was almost isolated from leaves as phyllosphere and phylloplane mycobiota respectively. This is probably because the leaf surface is exposed to rapidly fluctuating temperature and relative humidity, as well as repeated alternation between presence and absence of free moisture content dew. Also, the leaf itself is surrounded by a very thin laminar layer in which moisture emitted through stomata may be sequestered, thereby alleviating the water stress to which epiphytes are exposed **(Lindow and Brandl, 2003)**.

Temperature is one of the limiting factors in fungal growth and spread. In the present study, some fungi were isolated either at 19°C, such as *Humicola grisea*, *P. mirabile*, *Rhizoctonia solani*; or at 28°C, such as *Cheatomium brasiliense* and *Zopfiella latipes*. However the optimum temperature for *Rhizoctonia solani* growth ranged between 15 – 30°C **(Orozco-Avitia *et al.*, 2013)**.

In cluster analysis, the 5 sources (soil, rhizosphere, rhizoplane, phyllosphere and phylloplane) and two incubation temperatures (19° and 28ºC) were grouped based on total counts of fungal species (Figure 1). The analysis showed that, fungal species isolated from soil at 28ºC and rhizosphere at both 19º and 28ºC cluster closely together and they are the most similar to fungal community in soil at 19ºC. Also, fungal communities isolated from phyllosphere at both 19º and 28ºC were clustered together in the same group (C). The cluster analysis in figure (2) was used to compare different sources according to their number of genera and species. The cluster classify sources into four groups of which groups A and B are closely related, while, fungal genera and species isolated from soil at both 19º and 28ºC (group D) showed the least similarity with those isolated from other sources (Figure 2).

Figure 3a exhibited the distribution of genera is significantly different, for example *Acrophialophora* and *Setosphaeria* were closely related and showed significantly difference from *Alternaria*, *Fusarium* and *Trichoderma*. On the other hand, figure (3b) shows differences in composition of fungal genera isolated from different sources at 28ºC using detrended correspondence analysis (DCA). Interestingly, *Macrophomina* and *Rhizoctonia* are closely related and significantly different from *Alternaria, Fusarium* and *Sordaria*.

Table 1 Percentage total counts (%TC) and percentage frequency (%F) of fungi isolated from soil, rhizosphere, rhizoplane, phyllosphere and phylloplane of onion plants on PDA medium at both 19 and 28ºC. (n: the number of samples collected)

Fungal taxa	Soil (n = 26)				Rhizosphere (n = 20)				Rhizoplane (n = 20)				Phyllosphere (n = 20)				Phylloplane			
	19ºC		28ºC		19ºC		28ºC		19ºC		28ºC		19ºC		28ºC		19ºC		28ºC	
	%TC	%F	%TC	%F	%TC	%F	%TC	%F	%TC	%F	%TC	%F	%TC	%F	%TC	%F	%TC	%F	%TC	%F
Acremonium strictum W. Gams	0.1	14.5	0.8	19.2	0.99	15.00	0.74	10.00	-	-	0.08	5.00	0.1	5	0.2	5.0	-	-	0.3	5.0
Acrophalophora fusispora (Saksena) Samson	-	-	0.1	11.5	0.10	5.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alternaria alternata (Fries) Keissler	1.9	57.7	2.5	61.5	1.21	40.00	0.61	30.00	7.13	45.00	3.69	35.00	2.1	25	2.9	35.0	7.3	35.0	4.8	35
Aspergillus P. Micheli ex Link	93.5	100.0	50.2	100.0	54.49	100.00	63.32	100.00	25.63	90.00	39.64	100.00	33.4	100	38.5	95.0	36.5	100	49.7	100
A. carbonarius (Bainier) *et al.*	-	-	0.2	11.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. flavipes (Bainier & Sartory) Thom & Church)	-	-	-	-	-	-	0.12	5.00	-	-	-	-	-	-	-	-	-	-	-	-
A. flavus Link	32.2	34.6	2.0	46.2	0.78	20.00	0.65	10.00	2.19	10.00	2.26	15.00	2.4	25	2.1	25.0	4.3	20	4.3	25
A. fumigatus Fresenius	0.8	14.5	0.1	7.7	5.16	25.00	0.31	10.00					0.3	10	0.5	10.0	0.2	5.0	0.4	10.0
A. niger van Tieghem	24.1	100.0	34.9	100.0	44.93	95.00	55.70	95.00	21.71	90.00	35.57	100.00	27.1	100	29.9	95.0	28.2	100	40.7	100
A. niveus Blochwitz	-	-	-	-	0.26	5.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. ochraceus Wilhelm	24.2	19.2	0.2	19.2	0.47	30.00	0.07	5.00	-	-	0.68	10.00	2.1	25	4.2	25.0	1.7	5	3.0	15
A. oryzae (Ahlburg) Cohn	-	-	0.1	7.7	-	-	-	-	-	-	-	-	0.1	5	-	-	-	-	-	-
A. sydowii (Bainier & Sartory) Thom & Church	0.2	11.5	-	-	0.06	5.00	0.37	20.00	0.16	5.00	-	-	0.1	5	0.2	5.0	0.2	5	0.5	10
A. terreus Thom	5.6	73.1	12.0	84.6	1.96	30.00	3.81	55.00	1.41	20.00	0.75	15.00	0.9	15	1.4	30.0	1.6	20	0.5	15
A. ustus (Bainier) Thom & Church	5.6	3.8	0.0	3.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	5
A. versicolor (Vuillemin) Tiraboschi	0.8	30.8	0.8	30.8	0.88	30.00	2.29	50.00	0.16	5.00	0.38	15.00	0.3	5	0.3	5.0	0.1	5	-	-
Beauveria bassiana (Balsamo) Vuillemin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	5	-	-
Botryotrichum piluliferum Saccardo & Marchal	0.8	14.5	0.0	3.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Botrytis cinerea Persoon	0.5	14.5	-	-	2.49	15.00	-	-	0.39	10.00	-	-	9.5	30	1.8	25.0	11.8	35	2.0	15
Chaetomium Kunze	0.6	14.5	0.1	3.8	0.2	15.0	0.1	5.0	0.2	5.0	-	-	-	-	-	-	-	-	-	-
C. brasiliense Bat. & Pontual	-	-	0.1	3.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. globosum Kunze	0.6	14.5			0.2	15.0	0.1	5.0	0.2	5.0	-	-	-	-	-	-	-	-	-	-
Cladosporium Link	33.4	61.5	5.4	57.7	9.0	60.0	3.9	30.0	0.6	10.0	0.2	5.0	23.1	70	21.2	80.0	10.9	50	9.9	45
C. cladosporioides (Fresenius) de Vries	20.2	46.2	4.4	34.6	5.9	45.0	2.4	20.0	0.6	10.0	0.2	5.0	14.2	60	13.8	75.0	7.2	40	8.1	45
C. herbarum (Pers.) Link ex S. F. Gray	8.1	14.5	0.5	11.5	0.8	10.0	0.5	5.0	-	-	-	-	1.0	15	2.0	25.0	-	-	-	-
C. oxysporum Berkeley & Curtis	-	-	-	-	0.3	5.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. sphaerospermum Penzig	5.1	30.8	0.5	19.2	1.9	20.0	0.9	15.0	-	-	-	-	7.9	35	5.5	15.0	3.7	15.	1.8	10
Cochliobolus Drechsler	11.3	76.9	6.4	76.9	1.7	45.0	2.0	45.0	4.0	30.0	5.5	40.0	1.9	25	1.3	30.0	-	-	-	-
C. hawaiiensis Alcorn			0.0	3.8	0.1	5.0					0.3	5.0			0.1	5.0	-	-	-	-
C. lunatus R. Nelson & Haasis	6.1	42.3	1.2	50.0	0.3	20.0	0.3	15.0	1.6	15.0	2.6	25.0	0.4	15	0.3	10.0	-	-	-	-
C. spicifer Nelson	5.3	73.1	5.1	76.9	1.3	25.0	1.8	40.0	2.4	30.0	2.6	25.0	1.5	30	0.9	20.0	-	-	-	-
Cunninghamella echniulata (Thaxter) Thaxter	4.6	3.8	0.0	7.7			0.1	5.0	0.3	10.0	-	-					-	-	-	-
Emericella Berkeley & Broome	1.7	38.5	3.2	61.5	0.1	5.0	0.1	5.0	0.2	5.0	-	-	0.3	10	0.2	5.0	-	-	-	-
E. lata Subramanian	0.8	11.5	0.1	11.5	-	-	-	-	-	-	-	-					-	-	-	-
E. nidulans (Eidam) Vuillemin	0.3	23.1	1.7	50.0	-	-	-	-	0.2	5.0	-	-	0.3	10	0.2	5.0	-	-	-	-
E. rugulosa (Thom & Raper) Benjamin	0.6	30.8	1.4	34.6	0.1	5.0	0.1	5.0	-	-	-	-	-	-	-	-	-	-	-	-
Epicoccum nigrum Link	0.6	15.4	0.1	7.7	-	-	-	-	0.4	5.0	-	-	0.4	15	0.3	10.0	-	-	-	-
Eurotium amstelodami Mangin	-	-	0.1	7.7	-	-	-	-			-	-	-	-	-	-	-	-	-	-
Fusarium Link	13.5	76.9	8.2	69.2	5.3	50.0	2.2	35.0	20.0	90.0	12.5	70.0	2.7	25	3.1	40.0	3.7	25	2.8	30
F. chlamydosporum Wollenweber & Reinking	4.5	3.8	0.1	3.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F. equiseti (Corda) Saccardo	0.1	3.8	-	-	-	-	-	-	-	-	0.4	15.0	-	-	-	-	-	-	-	-
F. oxysporum Schlechtendal	4.0	69.2	6.3	61.5	5.3	50.0	1.7	35.0	18.9	90.0	11.4	70.0	1.6	20	1.8	25.0	3.7	25	2.8	30
F. oxysporum var redolens (Wollenw.) Gordon	-	-	0.0	3.8													-	-	-	-
F. solani (Martius) Saccardo	4.3	19.2	1.8	42.3	0.1	5.0	0.5	5.0	1.1	15.0	0.8	15.0	1.0	10	1.2	25.0	-	-	-	-
F. subglutinans (Wollenweber & Reinking) Nelson *et al*	-	-	0.0	3.8	-	-	-	-	-	-	-	-	-	-			-	-	-	-
F. tricinctum (Corda) Saccardo	0.5	3.8	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-
F. verticillioides (Saccardo) Nirenberg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	5.0	-	-	-	-
F. xylarioides Steyaert	0.1	3.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gliocladium roseum Bainier	0.2	15.4	0.0	3.8	0.1	5.0	0.1	5.0	-	-	-	-	0.4	10	-	-	0.4	10	0.1	5
Humicola grisea Traaen.	0.2	3.8	-	-	0.0	5.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Macrophomina phaseolina (Tassi) Goidanch	0.0	3.8	-	-	-	-	0.1	5.0			0.2	5.0	-	-	-	-	0.2	5	-	-
Mucor circinelloides van Tieghem	0.0	14.5	0.1	7.7	0.6	30.0	1.0	15.0	0.7	10.0	3.5	15.0	-	-	1.4	10.0	-	-	-	-
Mycothecium Tode	5.3	42.3	2.3	38.5	1.1	10.0	1.2	10.0	-	-	-	-	-	-	-	-	-	-	-	-
M. roridum Tode	2.9	11.5	0.8	7.7	0.7	5.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M. verrucoria (Albertini & Schweinitz) Ditmar	2.4	34.6	1.5	34.6	0.5	5.0	1.2	10.0	-	-	-	-	-	-	-	-	-	-	-	-
Nigrospora sphaerica (Saccardo) Mason	2.2	11.5	0.2	11.5	0.1	5.0	0.0	5.0	-	-	-	-	-	-	-	-	-	-	-	-
Penicillium Link	25.2	84.6	9.7	88.5	14.7	65.0	15.2	65.0	17.2	65.0	20.6	75.0	10.8	75	16.8	75.0	19.0	65	13.6	70
P. chrysogenum Thom	9.7	26.9	1.2	11.5	0.4	15.0	-	-	0.2	5.0	-	-	0.4	15	1.3	30.0	0.1	5	0.5	10
P. citrinum Thom	1.4	14.5	0.2	7.7	-	-	0.1	5.0	0.2	5.0	-	-	-	-	-	-	-	-	0.1	5
P. corylophilum Dierckx	0.1	3.8	0.1	11.5	0.2	10.0	0.2	15.0	0.3	5.0	0.3	5.0	0.3	10	0.3	10.0	0.7	10		
P. decumbens Thom	0.2	3.8	0.9	15.4	2.5	15.0	0.3	5.0	-	-	-	-	0.4	5	0.6	10.0				
P. duclauxii Delacroix	0.2	3.8	0.0	3.8	0.3	10.0	0.0	5.0	0.2	5.0	0.5	5.0	1.4	25	0.8	15.0			0.4	5
P. fellutanum Biourge	3.0	23.1	4.1	15.4	4.7	15.0	2.4	20.0	3.8	25.0	0.9	5.0	0.4	5	0.5	5.0	0.2	5		
P. funiculosum Thom	6.6	61.5	1.8	61.5	5.8	40.0	11.8	60.0	7.1	35.0	11.9	60.0	5.8	45.0	9.7	40.0	8.3	40	10.8	55
P. islandicum Sopp	3.6	3.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. mirabile Beliakova & Milko	0.1	3.8	-	-	0.1	10.0	-	-	-	-	-	-	-	-	-	-	0.1	5	-	-
P. oxalicum Currie & Thom	0.1	3.8	0.5	30.8	0.4	15.0	0.1	5.0	-	-	2.6	20.0	0.1	5.0	0.8	15.0	5.5	20	1.3	5

P. pinophilum Hedgcock	-	-	0.8	23.1	0.1	5.0			1.3	15.0	0.6	10.0	1.3	15.0	2.6	30.0	-	-	0.4	10
P. purpurgenum Stoll	0.1	3.8	0.1	7.7	0.2	10.0	0.1	5.0	1.2	5.0	2.0	10.0	0.3	10.0	0.3	5.0	-	-	-	-
P. waksmanii Zaleski	0.1	3.8	0.0	3.8	-	-	-	-	3.1	15.0	2.0	15.0	0.1	5.0	-	-	3.9	20	-	-
Phoma Saccardo	0.3	14.5	0.4	19.2	-	-	-	-	-	-	-	-	-	-	-	-	1.4	10	-	-
P. exigua Desmazieres			0.1	3.8	-	-	-	-	-	-	-	-	-	-	-	-			-	-
P. herbarum Westendorp	0.3	14.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. leveillei Boerema & Bollen	-	-	0.3	19.2	0.1	10.0	0.1	5.0	2.0	5.0	-	-	-	-	-	-	1.4	10	-	-
Rhizoctonia solani Kühn	-	-			0.1	15.0			1.3	15.0	-	-	-	-	-	-	2.1	10	-	-
Rhizopus stolonifer (Ehrenberg) Vuillemin	6.6	84.6	6.4	65.4	6.3	65.0	6.8	65.0	14.5	70.0	10.4	60.0	3.6	25.0	4.0	25.0	0.4	5	10.6	35
Setosphaeria rostrata Leonard	-	-	0.2	11.5	0.1	5.0	-	-	-	-	0.6	10.0	-	-	-	-	-	-	-	-
Sordaria fimicola (Roberge) Cesati & de Notaris	6.3	3.8	0.1	3.8	-	-	-	-	-	-	0.1	5.0	-	-	-	-	-	-	-	-
Stachybotrys Corda	0.2	15.4	0.2	15.4	0.1	10.0	0.2	15.0	-	-	-	-	0.4	15.0	-	-	-	-	-	-
S. chartarum (Ehrenberg) Hughes	-	-	0.1	7.7			0.1	10.0	-	-	-	-	0.4	15.0	-	-	-	-	-	-
S. elegans (Pidopl.) W. Gams	0.2	15.4	0.1	7.7	0.1	10.0	0.1	5.0	-	-	-	-			-	-	-	-	-	-
Stemphylium Wallroth	0.9	19.2	0.2	15.4	0.3	5.0	-	-	-	-	-	-	0.9	25.0	2.6	35.0	3.9	15	3.9	15
S. botryosum Wallroth	0.5	15.4	0.1	7.7	0.3	5.0	-	-	-	-	-	-	0.7	20.0	1.7	30.0	3.5	15	3.3	10
S. vesicarium (Wallr.) E.G. Simmons	0.4	3.8	0.1	7.7	-	-	-	-	-	-	-	-	0.1	5.0	0.9	10.0	0.5	5	0.6	5.
Sterile mycelia	-	-	0.1	11.5	-	-	-	-	-	-	-	-	0.1	5.0	-	-	-	-	-	-
Trichoderma Persoon	3.9	30.8	3.0	38.5	0.8	20.0	2.3	25.0	5.6	40.0	3.0	10.0	10.0	45.0	5.9	45.0	5.9	20	2.4	10
T. hamatum (Bonorden) Bainier	1.5	3.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T. harzianum Rifai	0.8	19.2	1.9	30.8	0.2	5.0	1.0	10.0	5.6	40.0	3.0	10.0	8.4	35.0	3.2	30.0	5.9	20	2.4	10
T. koningii Oudem	0.9	3.8	1.1	3.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T. longibrachiatum Rifai	0.7	3.8	0.0	3.8	0.7	20.0	1.2	15.0	-	-	-	-	1.6	15.0	2.7	20.0	-	-	-	-
Zopfiella latipes (N. Lundq.) Malloch & Cain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	10
Total counts	1863876	552360			679945		572980		1276		1327		133500		131300		811		944	
No. of genera 32	25	26			23.0		19.0		16.0		13.0		15		14		14		12	
Number of species 79	58	60			47.0		40.0		30.0		28.0		37		36		28		25	

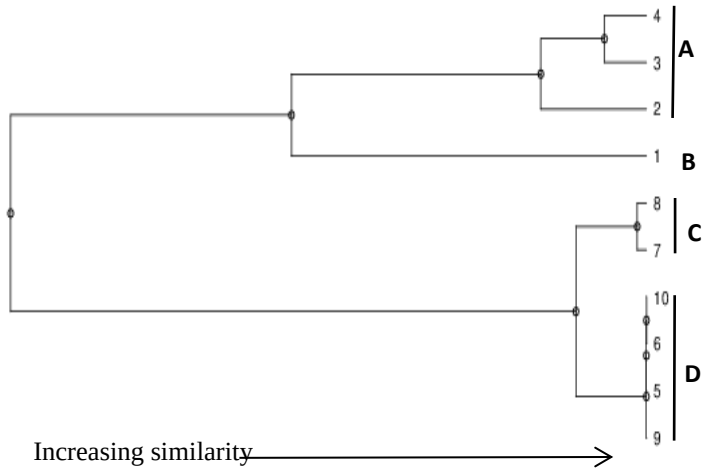


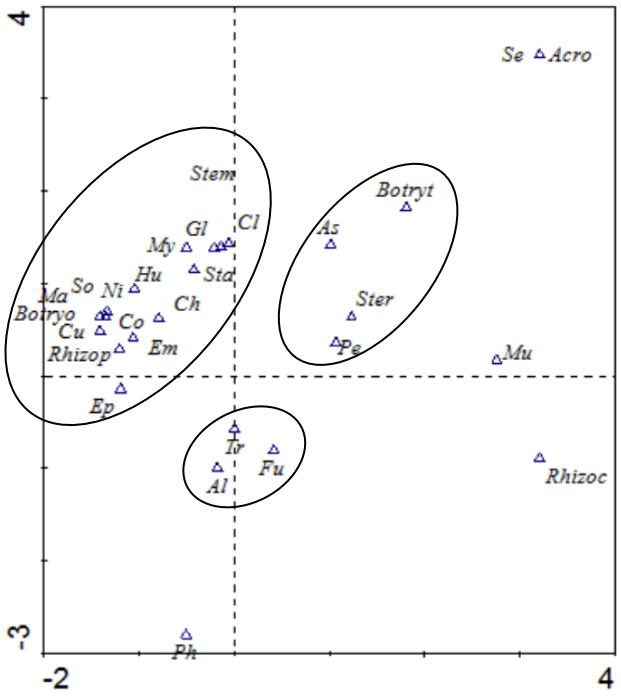
Figure 1 Cluster analysis of 5 sources; soil at 19°C incubation temperature (1), soil at 28°C (2), rhizosphere at 19°C (3), rhizosphere at 28°C (4), rhizoplane at 19°C (5), rhizoplane at 28°C (6), phyllosphere at 19°C (7), phyllosphere at 28°C (8), phylloplane at 19°C (9) and phylloplane at 28°C (10) based on the similarity of their fungal communities using species total counts. The sources cluster into four major groups (A to D). Sources of group C are similar to those of group D, while groups A and B have low similarity with groups C and D.

New record species in Egypt

A fungal species was recorded in this study from phylloplane (green leaves of onion plant at 28ºC) for the first time in Egypt, namely *Zopfiella latipes* . It was isolated from samples and accounting 0.32% of total phylloplane fungi (Table).
***Zopfiella latipes* (Lundqvist) Malloch and Cain 1971**
Synonym: *Tripterospora latipes* **Lundqvist, 1969**

Macroscopic features: On PDA and glucose-Czapek's agar, colony grows fast, attaining 9 cm in diameter after 10 days at 28ºC, greyish brown to olive brown, velutinous; reverse dark brown.

Microscopic features: Ascomata pale greyish brown or dark brown to black, globose or subglobose, superficial, non-ostiolate, irregularly dehiscing, 120-205 µm in diameter, and covered with hyphae. Peridium semitransparent, with soft skin, composed of three or four layers of irregular or angular thin-walled pseudoparanchymatous cells (Figure 4 A,B). Asci are 8-ascosporous, thin walled, clavate, broadest in the middle, 80-120×14-18 µm, apically truncate (Figure 4C). Ascospores biseriate, ellipsoidal, globulate, becoming one-septate in the lower third, slightly constricted at the septum, 2-celled; large upper cell 16-25 x 12-15 µm, ellipsoidal, apex conical or umbonate, base truncate, olivaceous to brown, thin-walled, smooth, with a subapical germ pore, 1 µm in diameter, lower cell small, 4-8 x 3.5-7µm, broadly cylindrical, apex truncate, base broadly rounded, hyaline **(Malloch and Cain, 1971; Furuya and Udagawa, 1973)** (Figure 4B-D). *Zopfiella latipes* belongs to Phylum: Ascomycota, Subdivision: Pezizomycotina, Class: Sordariomycetes, Subclass: Sordariomycetidae, Order: Sordariales, Family: Lasiosphaeriaceae.
It was isolated from soil and wood immersed in sea water **(Malloch and Cain, 1971)**, from freshwater habitats in Florida Peninsula **(Raja *et al.*, 2009)**, from three mangrove plants in India **(Manimohan *et al.*, 2011)**.



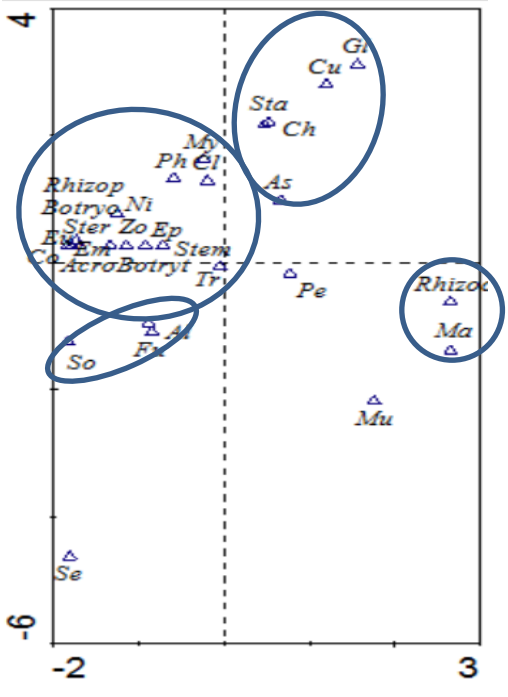


Figure 3a (left). Testing of differences in fungal genera composition of soil, roots and green leaves of onion isolated at 19ºC; DCA ordination diagram showing the position of fungal genera in different sources; *Acrophalophora* (Acro), *Alternaria* (Al), *Aspergillus* (As), *Botryotrichum* (Botryo), *Botrytis* (Botryt), *Chaetomium* (Ch), *Cladosporium* (Cl), *Cochliobolus* (Co), *Cunninghamella* (Cu), *Emericella* (Em), *Epicoccum* (Ep), *Fusarium* (Fu), *Gliocladium* (Gl), *Humicola* (Hu), *Macrophomina* (Ma), *Mucor* (Mu), *Nigrospora* (Ni), *Penicillium* (Pe), *Phoma* (Ph), *Rhizoctonia* (Rhizoc), *Rhizopus* (Rhizop), *Setosphaeria* (Se), *Sordaria* (So), *Stachybotrys* (St), *Stemphylium* (Stem), Sterile mycelia (Ster) and *Trichoderma* (Tr).

Figure 3b (right). Testing of differences in fungal genera composition of soil, roots and green leaves of onion isolated at 28ºC; DCA ordination diagram showing the position of fungal genera in different sources; *Acrophalophora* (Acro), *Alternaria* (Al), *Aspergillus* (As), *Botryotrichum* (Botryo), *Botrytis* (Botryt), *Chaetomium* (Ch), *Cladosporium* (Cl), *Cochliobolus* (Co), *Cunninghamella* (Cu), *Emericella* (Em), *Epicoccum* (Ep), *Eurotium* (Eu), *Fusarium* (Fu), *Gliocladium* (Gl), *Macrophomina* (Ma), *Mucor* (Mu), *Mycothecium* (My), *Penicillium* (Pe), *Phoma* (Ph), *Rhizoctonia* (Rhizoc), *Rhizopus* (Rhizop), *Setosphaeria* (Se), *Sordaria* (So), *Stachybotrys* (St), *Stemphylium* (Stem), Sterile mycelia (Ster) and *Trichoderma* (Tr).

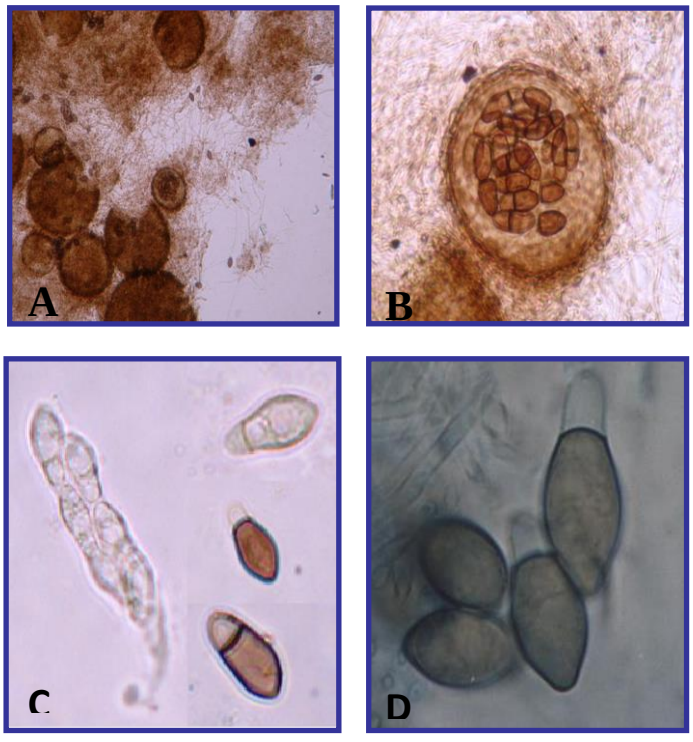


Figure 4 *Zopfiella latipes*: Non ostiolate ascoma (A and B); Clavate asci, with 8-ascosporous & mature and immature ascospores (C); 2-celled ascospores (D)

CONCLUSION

The fungal compositions of soil, root and leaf surfaces fungi associated with onion plant were determined, resulting in collecting 79 species belonging to 32 genera, of which *Zopfiella latipes* was recorded during this investigation for the first in Egypt. The fungal species isolated from soil at 28ºC and rhizosphere at 19º and 28ºC are similar to fungal community in soil at 19ºC. Also, fungal communities isolated from phyllosphere at both 19º and 28ºC are basically similar.

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