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EVALUATION OF PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF *ALTERNARIA* SPECIES ISOLATED FROM SOIL IN ASSIUT GOVERNORATE, EGYPT, IN ADDITION TO DICHOTOMOUS KEY TO THE ENCOUNTERED SPECIES

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Mycobiota of 25 soil samples, collected from Botanical garden of Botany and Microbiology Department, Faculty of Science, Assiut University, were monitored on acidified weak potato dextrose agar (AWPDA), dichloran chloramphenicol peptone agar (DCPA), dichloran glycerol agar (DG-18) and potato carrot agar with manganese (PCA-Mn) media at 25 ºC. One hundred and twenty-two species belong to 47 genera were obtained. *Aspergillus* was the most predominant genus on all media and *A. niger* was superior on AWPDA, DG18 and PCA-Mn. *Fusarium* was the runner up, followed by *Alternaria, Curvularia, Talaromyces, Penicillium* and *Stachybotrys*. Fifteen isolates of six species of *Alternaria* were subjected to some physiological and biochemical tests. The statistically analyzed results showed that, all isolates could significantly grow on ammonium tartarate, ammonium oxalate and glycine, while 14, 13 and 11 isolates wer able to grow on citric acid, lactic acid and sodium nitrite, respectively. *Alternaria tenuissima* and *A. angustiovoidea* were highly base producers from ammonium tartarate, while, the maximum base production on glycine was observed by *A. angustiovoidea* and *A. arborescens*. All isolates enabled to grow on creatine-sucrose and *A. arborescens* produced the strongest color, whereas the remaining isolates were negative (11 isolates) to moderate (3). On the other hand, *A. chlamydospora* and *A. angustiovoidea* showed maximum growth on mannitol and tannin-sucrose, respectively. Besides, the growth on 15 % NaCl-Cz was distinctive for 60 % and 50 % of *A. alternata* and *A. angustiovoidea* isolates, respectively.

Keywords: *Alternaria*, biochemical, dichotomous key, mycobiota, physiological, soil.

1. INTRODUCTION

Soil-borne fungi have large capabilities to break down all kinds of organic substances and decompose soil components, due to their potential to produce a wide variety of extracellular enzymes [1, 2]. In Egypt, soil fungi have been extensively studied [3-11]. However, these fungi still require further studies for their recovering since soil is very rich habitat with fungi. *Alternaria* is one of the world wide distributed genera including saprobic, endobiotic and pathogenic species occupying a wide spectrum of

environments and substrates such as soil, air, seeds, plants, agricultural products and others. Several studies worldwide, concerned with the descriptions and revision of *Alternaria* and its related genera [12-18] resulted in growing number of new species. Taxonomical studies of *Alternaria*, based upon the morphological characteristics, have summarized in [19], in which 276 *Alternaria* species were recognized.

Recently, there has been a huge number of the progressive publications focusing on *Alternaria* taxonomy to correct and for rapid identification of *Alternaria* species, which would be of great value for researchers and medical mycologists [20-22]. Apparently, morphological and physiological differentiations among Alternaria species, in addition to phylogenetic studies, have been important concepts in the field of taxonomy. Therefore, the aim of the current study was to estimate fungal diversity of soil, determine morphological criteria and evaluate some physiological and biochemical activities of isolated *Alternaria* species.

2. MATERIALS AND METHODS

2.1. Isolation of fungi

Dilution plate method [23] was used for isolation of fungi from 25 cultivated soil samples collected from the Botanical garden of Botany and Microbiology Department, Faculty of Science, Assiut University, Assiut Governorate, Egypt. Four media were used; acidified weak potato-dextrose agar (AWPDA) [24], Dichloran chloramphenicol peptone agar DCPA [25], Dichloran glycerol agar DG18 [26], and potato carrot agar with manganese (PCA-Mn) [27]. One ml of appropriate dilution of each sample was transferred to Petri dish (9 mm), then 20 ml of the isolation media were pored into the petri dishes. The plates were then incubated at 25 °C for 14 days. Colony forming units (CFUs) were calculated per 1 g dry soil for all samples.

2.2. Phenotypic identification of fungi

The isolated genera and species were identified phenotypically, based on their macro- and microscopic features, following the available keys and descriptions of [6, 19, 28-37].

2.3. Morphological studies of *Alternaria* species

Macro- and microscopic features of fifteen isolates belong to 6 species of *Alternaria* collected in the current study were assessed on DCPA, PCA and V8-juice agar (V-8) media at 22 °C under a light/dark cycle of 8/16 h. For slide preparation, a rectangular block of agar (0.5 × 2.0 cm) was removed within the margin of the colony at the 5th day, then re-incubated at 22 °C. The colony development and sporulation patterns were monitored and slide preparations were examined at the 7th day. Cultures were mounted with a drop of 60 % lactic acid for observation of sporulation patterns and conidial chains as recommended by Simmons [19, 38]. Colony characteristics (colony diameter, appearance, concentric rings, and sporulation on cut-agar surface after 3 days), as well as microscopic features (conidial chain formation after 7 days incubation, conidiophores and conidial characteristics) were evaluated. The six species involved in the current study were described and identified based on their morphological characteristics.

2.4. Physiological and biochemical tests

2.4.1. Growth and base production from different carbon and nitrogen sources

The ability of *Alternaria* isolates (15) to grow and produce base from ammonium tartarate, citric acid and lactic acid (as sole carbon sources), as well as ammonium oxalate, glycine and sodium nitrite (as sole nitrogen sources) were studied. pH of the media was adjusted to 4.5 before autoclaving; the plates were incubated for 7 days at 25 ºC. Growth diameter and color change of bromocresol purple from yellow (in acidic medium) to purple (in alkaline medium) were recorded visually and expressed as high (H), moderate (M) and weak (W) intensity [39].

2.4.2. Growth at different temperatures and pH values

The isolates were grown on Czapek's agar medium [40] at different temperatures (5, 35 and 45 °C) and pH values (5, 9 and 11). Colony diameters were measured after 7 days of incubation.

2.4.3. Growth on different media

Growth rates of the fifteen isolates of *Alternaria* were assessed after 7 days at 25 ºC on DCPA, DG-18, 25 %–Glycerol nitrate agar [G25N [41],

15 % NaCl–Cz, 50 % Sucrose–Cz, creatine–sucrose agar media [42], ammonium salts agar (mannitol agar) [43] and tannin sucrose agar media [TAN, [44].

2.5. Screening of *Alternaria* species for their enzymes production

2.5.1. Peroxidase

The fifteen isolates were inoculated on malt extract agar [MEA, [45] plates, and incubated for 7 days at 25 °C. A five mm diameter-well in the agar medium was filled with 100 µl of freshly prepared 1 % aqueous solution of pyrogallic acid. The development of golden yellow to brown color indicated positive peroxidase activity [46].

2.5.2. Pyrocatechol oxidase

The potentiality of fifteen *Alternaria* isolates for production of pyrocatechol oxidase enzyme was tested on MEA medium supplemented with 0.17 % tannic acid [47]. The plates were then incubated for 10 days at 25 °C. Pyrocatechol oxidase production was monitored as the formation of dark brown zone around the colonies.

2.5.3. Urease

To determine the capability for urease production, the isolates were grown on urease medium [48] and incubated for 7 days at 25 °C. The positive results were expressed as the formation of red or deep pink color around the colonies.

2.6. Statistical analysis

Differences between means were subjected to SPSS program software version 21.0 (SPSS Inc., Chicago, USA) for analysis by One-way ANOVA was carried out using. The *P* values less than 0.05 was considered statistically significant.

3. RESULTS

One hundred and twenty-two species appertaining to 47 genera were isolated from soil on AWPDA (34 genera and 81 species), DCPA (31 and 71), DG18 (26 and 67) and PCA-Mn (35 and 75). Although DG18 medium recorded the highest total count (11236 CFU/g soil), it ranked the last in the number of genera (26) and species (67). The most common genera were

Aspergillus (32 species), *Fusarium* (11 species), *Alternaria*, *Curvularia* and *Talaromyces* (6 species each), *Penicillium* and *Stachybotrys* (5 species each). The remaining genera were represented by only one or two species (Table 1). The most common species in the current study were *Aspergillus brasiliensis, A. flavus, A. niger, A. terreus, Alternaria alternata, A. tenuissima, Curvularia lunata, C. spicifera,* C. *tsudae, Fusarium chlamydosporum, F. oxysporum, F. solani* and *P. chrysogenum.*

3.1. Fungi isolated from soil

3.1.1. On AWPDA

Thirty-four genera represented by 81 species were isolated and identified from cultivated soil on AWPDA at 25 ºC, from which *Aspergillus* ranked the first (46.5 % of total fungi and 100 % of total samples), followed by *Fusarium* (14.01 % and 84.0 %), and *Penicillium* (9.79 % and 40.0 %). *Aspergillus niger* was the most predominant species (14.34 % of total fungi and 92.0 % of total samples tested), followed by *A. terreus* (10.28 % and 60.0 %), and *Fusarium solani* (9.55 % and 64.0 %) (Table 1).

3.1.2. On DCPA

Thirty-one genera and 71 species were recovered, from which *Aspergillus*, representing 55.94 % of total fungal counts and 96.0 % of total samples, was the most common genus, followed by *Fusarium* (18.30 % and 96.0 %), *Penicillium* (11.04 % and 32.0 %) respectively. Regarding to fungal species, *Aspergillus flavus* (21.87 % of total fungi and 72.0 % of total samples), *A. niger* (13.14 % and 84.0 %), *A. terreus* (8.88 % and 64.0 %), *Fusarium solani* (11.98 % and 72.0 %) and *Penicillium chrysogenum* (7.51 % and 16.0 %) respectively represented the most common species (Table 1).

3.1.3. On DG18

Sixty-seven species related to 26 genera were obtained from 25 cultivated soil samples on DG18 medium. Similar to the above media, *Aspergillus* showed the highest total count and frequency (62.90 % and 100.0 %) and *Penicillium* (17.76 % and 60.0 %) ranked the second. Of these, *Aspergillus niger* (19.58 % and 96.0 %), *A. flavus* (13.03 % and 72.0 %), *A. terreus* (9.72 % and 64.0 %), *A. nidulans* (6.37 % and 60.0 %) were the highest frequent species (Table 1).

3.1.4. On PCA-Mn

Thirty-five genera and 75 species were collected, from which, *Aspergillus* and *Fusarium* (56.58 % and 16.32 % of total fungi and 92.0 % and 72.0 % of total samples) respectively were the most predominant genera. The most abundant species were *Aspergillus niger* (13.49 % and 68.0 % of total fungi and total samples respectively), followed by *A. flavus* (12.33 % and 48.0 %) and *Fusarium solani* (12.10 % and 56.0 %) (Table 1).

Table 1: Total counts × 10^2 (TC, calculated for each species per 1 g dry soil in all samples) and percentage frequency of occurrence (% F) of fungi isolated from 25 cultivated soil samples on AWPDA, DCPA, DG18 and PCA-Mn at 25 °C.

Fungal species	AWPDA		DCPA		DG18		PCA-Mn	
	TC	%F	TC	%F	TC	%F	TC	%F
Acremonium *roseolum*							28	8
Acrophialophora *fusispora*	16	4	152	20	32	4	32	8
Albifimbria *verrucaria*	140	24	24	12	48	16	56	28
Alternaria	176	28	64	16	84	16	92	24
A. alternata	76	16	24	8	36	8	24	8
A. angustiovoidea			8	4			8	4
A. arborescens	8	4						
A. chartarum	8	4					16	4
A. chlamydospora							28	12
A. tenuissima	84	16	32	8	48	8	16	4
Ascotricha *guamensis*	4	4						
Aspergillus	4540	100	6200	96	7068	100	4076	92
A. amstelodami					148	28		
A. aureolatus	120	12	12	4	16	4	324	16
A. brasiliensis	56	12	136	20	172	20	148	20
A. candidus	800	4	8	4				
A. carbonarius	2800	4	8	4	32	4		
A. carneus					24	8		
A. flavipes	16	8	4	4				
A. flavus	452	40	2424	72	1464	72	888	48
A. fumigatus	228	20	4	4	40	16	384	28
A. lanosus	32	4	52	8	44	12		
A. latus	40	12	8	4			12	8
A. melleus	16	4			40	4	16	4
A. neoniveus	12	8			4	4		
A. nidulans	288	24	40	16	716	60	32	12
A. niger	1400	92	1456	84	2200	96	972	68
A. ochraceus	284	32	668	28	796	40	184	28
A. parasiticus							4	4
A. quadrilineatus			20	4			28	4
A. ruber	8	4	24	8	80	12	4	4
A. rugulosus							8	4
A. sclerotiicarbonarius					52	4		
A. sclerotiorum			88	4				
A. spinulosporus	24	4					2	4
A. stellatus	32	4	24	8				
A. sulphureus			4	4				
A. sydowii	28	16	24	12	36	16	8	4
A. tamarii			96	16	28	8		
A. terreus	1004	60	984	64	1092	64	620	30

Fungal species	AWPDA		DCPA		DG18		PCA-Mn	
	TC	%F	TC	%F	TC	%F	TC	%F
A. tubingensis					16	4		
A. unguis	396	28	88	12			308	16
A. ustus	68	28	12	12	24	12	112	28
A. versicolor			16	8	44	16		
Bipolaris *sorokiniana*	12	4						
Bisifusarium *dimerum*			4	4				
Chaetomium	4	4	120	12	8	4	12	4
C. globosum							12	4
C. pululiferum	4	4	120	12	8	4		
Cladosporium	336	24	124	20	624	48	72	12
C. cladosporioides	32	8	36	8	288	20	4	4
C. sphaerospermum	304	20	88	12	336	40	68	8
Clonostachys *rosea*	24	4	4	4	4	4	32	20
Cunninghamella *echinulate*			4	4	4	4	20	4
Curvularia	708	72	504	52	240	36	436	60
C. brachyspora					4	4		
C. eragrostidis							4	4
C. hawaiiensis	28	4			4	4	28	8
C. lunata	228	24	188	20	8	8	172	20
C. spicifera	444	52	296	44	204	36	220	40
C. tsudae	8	4	20	8	20	8	12	4
Didymella	172	20	40	12	20	12	24	16
D. glomerata	172	20	40	12	20	12	16	12
D. pomorum							8	4
Epicoccum	8	4	8	4	16	12	4	4
E. layuense	8	4						
E. nigrum			8	4	16	12	4	4
Exserohilum *rostratum*	12	4					24	8
Fusarium	1368	84	2028	96	680	68	1176	72
F. acuminatum	12	4					20	4
F. anthophilum					24	4		
F. chlamydosporum	84	20	44	12	24	12	88	20
F. fujikuroi	32	8	16	8				
F. incarnatum	44	16	188	20	100	20	8	4
F. oxysporum	184	36	404	36	232	36	120	16
F. proliferatum	8	8			16	4	48	4
F. roseum	16	8			60	4		
F. solani	932	64	1328	72	224	36	872	56
F. sulphureum			12	8				
F. tricinctum	56	4	36	8			20	4
Graphium *penicillioides*							16	4
Humicola	32	64	64	64	8	16		
H. fuscoatra	16	8						
H. grisea	16	8	64	16	8	4		
Juxtiphoma *eupyrena*	148	20	28	12	4	4	168	4
Lichtheimia *corymbifera*	24	4	8	4				
Macrophomina *phaseolina*			4	4			28	4
Microascus	4	4	32	4	20	4		
M. brevicaulis			32	4	20	4		
M. cinereus	4	4						
Monodictys	4	4	4	4			4	4
M. glauca			4	4				
M. putredinis	4	4					4	4
Mucor	124	4	80	8	8	8	56	4
M. circinellioides					4	4		
M. hiemalis	124	4	80	8	4	4	56	4
Nigrospora *oryzae*	20	8	4	4	20	8	12	4
Ochroconis *tshawytschae*	8	4					12	4

Fungal species	AWPDA		DCPA		DG18		PCA-Mn	
	TC	%F	TC	%F	TC	%F	TC	%F
Paecilomyces	4	4	8	4	32	8		
P. fulvus	4	4						
P. variotii			8	4	32	8		
Papulaspora *immersa*	28	8					4	4
Paramyrothecium *rorridum*	32	12					60	12
Paraphoma *fimeti*							16	4
Parascedosporium *putredinis*	52	4	8	4			40	4
Penicillium	956	40	1224	32	1996	60	44	8
P. aurantiogriseum	400	20	16	4	440	28		
P. chrysogenum	376	16	832	16	528	36	36	4
P. citrinum	40	8	44	12	504	8		
P. glabrum							8	4
P. oxalicum	140	8	332	4	524	8		
Phoma *leveille*			36	8				
Pleurostoma *richardsiae*					4	4		
Pseudopithomyces *chartarum*	20	4						
Purpureocillium *lilacinum*			16	8	4	4		
Rhizopus	76	12			88	16	104	28
R. microsporus							4	4
R. stolonifer	76	12			88	16	100	24
Sarocladium	216	24	64	24	28	12	28	20
S. kiliense	28	8	4	4				
S. strictum	188	16	60	20	28	12	28	20
Scopulariopsis *brumptii*			16	4			8	4
Scolecobasidium *constrictum*							20	4
Scytalidium *lignicola*	60	12	32	12	16	8	24	8
Stachybotrys	216	36	76	20	36	12	300	48
S. chartarum	156	36	68	16	16	12	220	44
S. echinatus	4	4					12	4
S. elegans	52	4	8	4			68	16
S. havanensis					20	4		
S. levisporus	4	4						
Stemphylium *vesicarium*	16	4						
Talaromyces	120	20	104	24	144	24	164	24
T. duclauxii	12	4	68	16	28	8	12	4
T. funiculosus	60	8	16	8	28	8	44	8
T. helicus					4	4		
T. islandicus							4	4
T. pinophilus	4	4			4	4		
T. purpureogenus	44	12	20	4	80	12	104	12
Trichoderma *harzianum*	84	16					8	8
Verticillium *nubilum*							4	4
Total count	**9764**		**11084**		**11236**		**7204**	
No of genera: 47	**34**		**31**		**26**		**35**	
No of species: 122	**81**		**71**		**67**		**75**	

TC: Total count of colony forming units (calculated per 25 g dry soil samples).
%F: Percentage incidence of species per 25 samples.

3.1.5. Effect of different carbon and nitrogen sources on the growth of and base production from *Alternaria* species.

All the fifteen *Alternaria* strains tested could significantly grow on ammonium tartarate, ammonium oxalate and glycine ($P < 0.05$), while 14, 13 and 11 strains could grow on citric acid, lactic acid and sodium nitrite,

respectively. *A. angustiovoidea* and *A. tenuissima* were highly base producers from ammonium tartarate (Figure 1; Table 2).

3.1.6. Effect of different pH values on growth of *Alternaria* species.

The current results showed that the maximum growth of *A. alternata*, *A. chlamydospora* and *A. tenuissima* was observed at pH 5.0, while pH 9.0 and pH 11.0 were the best for the growth of *A. angustiovoidea* and *A. chlamydospora* respectively (Figure 2; Table 3).

3.1.7. Effect of different temperatures on growth of *Alternaria* species

The current results showed that the critical temperature degrees varied in different *Alternaria* strains as all *Alternaria* species could significantly ($P < 0.05$) grow at 5 ºC and 35 ºC, and no significantly growth was recorded at 45 ºC. The maximum growth of *A. alternata*, *A. angustiovoidea*, *A. chlamydospora* and *A. tenuissima* was observed at 35 ºC while *A. chartarum* at 5 ºC (Figure 3; Table 3).

3.1.8. Growth on different media

The current results showed that all the tested isolates could significantly ($P < 0.05$) grow on all the tested growth media depending on the behavior of the fungal strain, environmental and nutritional conditions. All the tested media were significantly varied ($P < 0.05$) for all *Alternaria* isolates, although the maximum growth has observed on creatine-sucrose, followed by TAN, G25N agar. On the other hand, *A. alternata* (3 isolates) and *A. angustiovoidea* (1 isolate) could grow on 15 % NaCl-Cz. All isolates could grow on creatine-sucrose and *A. arborescens* had the highest capability to change the medium color, while the remaining isolates were negative to moderate. *A. chlamydospora* showed a significant maximum growth on mannitol and *A. angustiovoidea* on tannin-sucrose (Figure 4; Table 4).

3.1.9. Enzyme production

The current results revealed that, 9, 13 and 14 (out of 15) tested *Alternaria* isolates were able to produce, peroxidase, pyrocatechol oxidase and urease enzymes respectively with variable degrees (Table 5). Moreover, other isolates belonging to *A. angustiovoidea* (2 isolates) showed maximum production of pyrocatechol oxidase and urease.

Table 2: Colony diameter (in mm) and base production within the identified species of *Alternaria* as shown by cultures on different carbon and nitrogen sources.

***Alternaria* species (IN)**	**Carbon source**			**Nitrogen source**		
	Ammonium tartrate	**Citric acid**	**Lactic acid**	**Ammonium oxalate**	**Glycine**	**Sodium nitrite**
A. alternata (5)	45.2 ± 3.2	44.7 ± 2.6	23.3 ± 5.8	36.1 ± 5.5	42.2 ± 3.2	18.6 ± 12.9
A. angustiovoidea (2)	34.2 ± 0.98	44.8 ± 3.1	21.8 ± 6.96	32.0 ± 1.55	43.3 ± 1.86	27.3 ± 6.0
A. arborescens (1)	31.7 ±1.5	42.7 ± 2.5	0.0	31.7 ± 1.5	44.0 ± 3.6	0.0
A. chartarum (1)	25.7 ± 5.1	20.0 ± 1.0	9.3 ± 1.15	24.7 ± 5.5	33.3 ± 3.0	27.7 ± 2.5
A. chlamydospora (1)	42.7 ± 2.5	47.3 ± 2.5	25.3 ± 2.5	32.3 ± 2.5	54.7 ± 4.5	0.0
A. tenuissima (5)	36.5 ± 5.4	31.1 ± 16.15	18.2 ± 9.8	36.3 ± 3.86	39.6 ± 4.9	20.3 ± 17.6
F-test (Significance)	*	*	*	*	*	*

(IN)= Isolates numbers; F-test: (*) significant

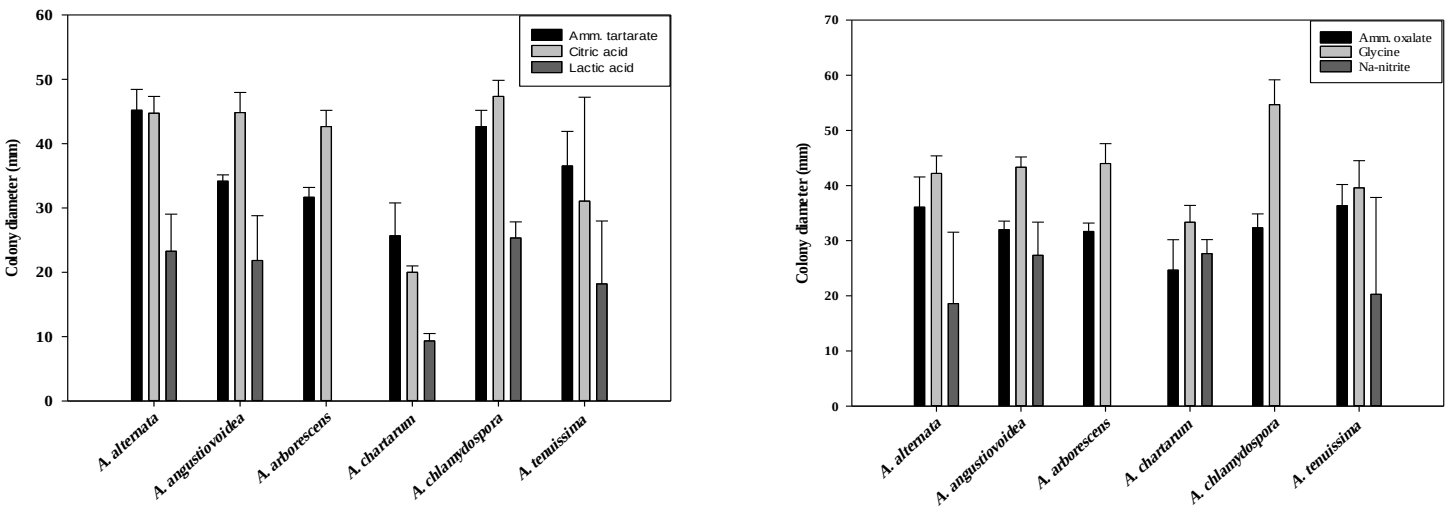

Figure 1: Effect of different carbon and nitrogen sources on the growth of *Alternaria* species

Table 3: Statistical analysis of colony diameter (in mm) within the identified species of *Alternaria* on different pHs and temperatures.

***Alternaria* species (IN)**	**pH**			**Temperature °C**	
	5	**9**	**11**	**5**	**35**
A. alternata (5)	65.3 ± 13.0	66.3 ± 4.98	46.9 ± 11.9	18.9 ± 6.5	26.5 ± 6.15
A. angustiovoidea (2)	50.5 ± 10.2	62.0 ± 6.0	46.0 ± 2.0	10.5 ± 0.54	22.8 ± 3.2
A. arborescens (1)	54.3 ± 6.0	50.7 ± 1.1	42.3 ± 2.1	0.0	0.0
A. chartarum (1)	41.3 ± 2.1	33.0 ± 3.0	30.7 ± 3.0	13.0 ± 3.0	6.7 ± 2.3
A. chlamydospora (1)	75.7 ± 5.1	69.3 ± 3.0	80.0 ± 5.0	7.7 ± 2.1	25.0 ± 6.0
A. tenuissima (5)	66.9 ± 3.8	64.9 ± 4.8	52.7 ± 7.4	11.4 ± 6.7	24.1 ± 4.6
F-test (Significance)	*	*	*	*	*

(IN)= Isolates number; F-test: (*) = significant

Table 4: Statistical analysis of colony diameter (in mm) using different growth media within the identified species of *Alternaria* on different growth media at 25 ºC.

Alternaria species (IN)	DCPA	DG18	G25N	15% NaCl-Cz	50% Cz-S	CREA	Mannitol	TAN
Alternaria alternata (5)	13.7 ± 1.7	30.5 ± 2.4	37.1 ± 2.7	1.8 ± 2.3	35.9 ± 3.3	64.3 ± 4.9	28.9 ± 4.2	39.4 ± 3.6
A. angustiovoidea (2)	19.2 ± 3.4	25.8 ±7.5	27.3 ± 6.4	4.5 ± 1.2	37.5 ± 1.2	74.5 ± 4.3	33.5 ± 2.0	52.3 ± 2.4
A. arborescens (1)	12.0 ± 2.0	32.3 ±2.1	26.7 ± 1.5	NS	35.7 ± 1.2	72.0 ± 2.0	30.7 ± 4.0	43.3 ± 2.9
A. chartarum (1)	13.3 ± 2.1	28.3 ±1.5	25.7 ± 2.1	NS	35.0 ± 2.0	36.0 ± 2.0	23.0 ± 2.0	38.3 ± 2.1
A. chlamydospora (1)	33.7 ± 3.2	45.0 ± 5.0	29.7 ± 2.3	NS	24.7 ± 1.5	75.0 ± 5.0	34.7 ± 2.5	26.7 ± 4.2
A. tenuissima (5)	16.8 ± 3.7	28.9 ± 4.0	33.6 ± 7.4	NS	38.4 ± 3.5	70.9 ± 4.7	26.0 ± 4.3	42.1 ± 5.9
F-test (Significance)	**	**	**	**	**	**	**	**

(IN) = Isolates number; F-test (**) = significant; NS= Not significant

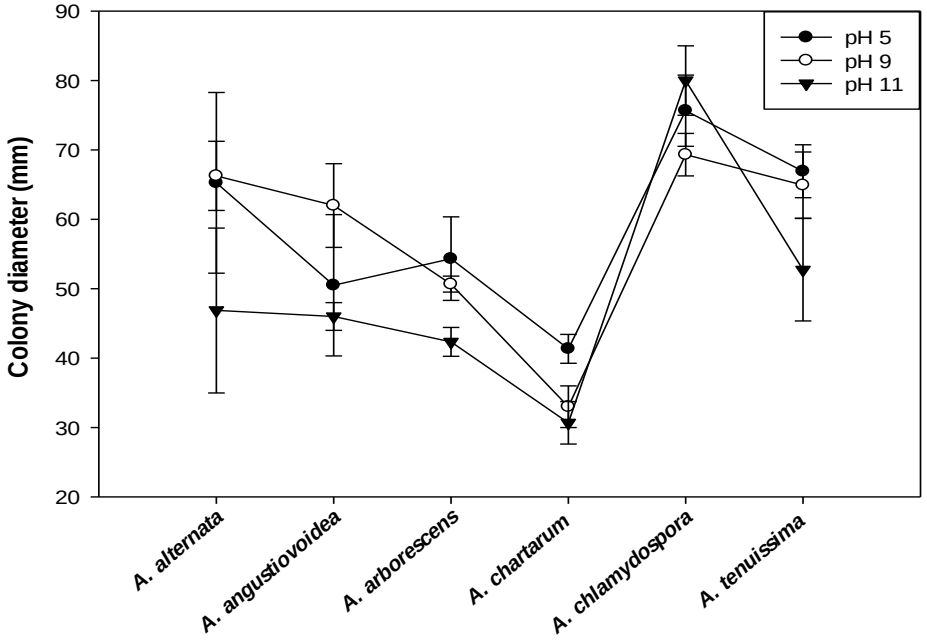

Figure 2: Effect of pH on the growth of *Alternaria* species

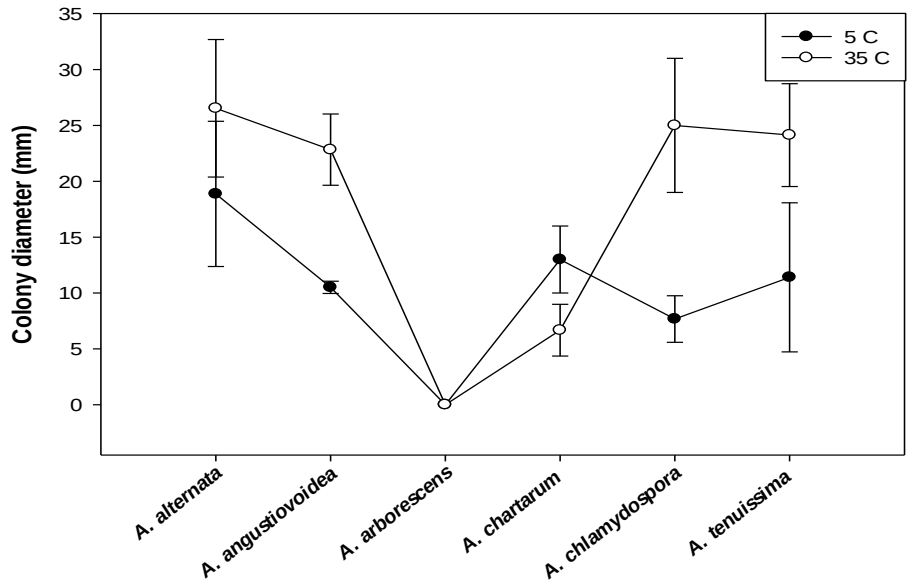

Figure 3: Effect of temperature on the growth of *Alternaria* species

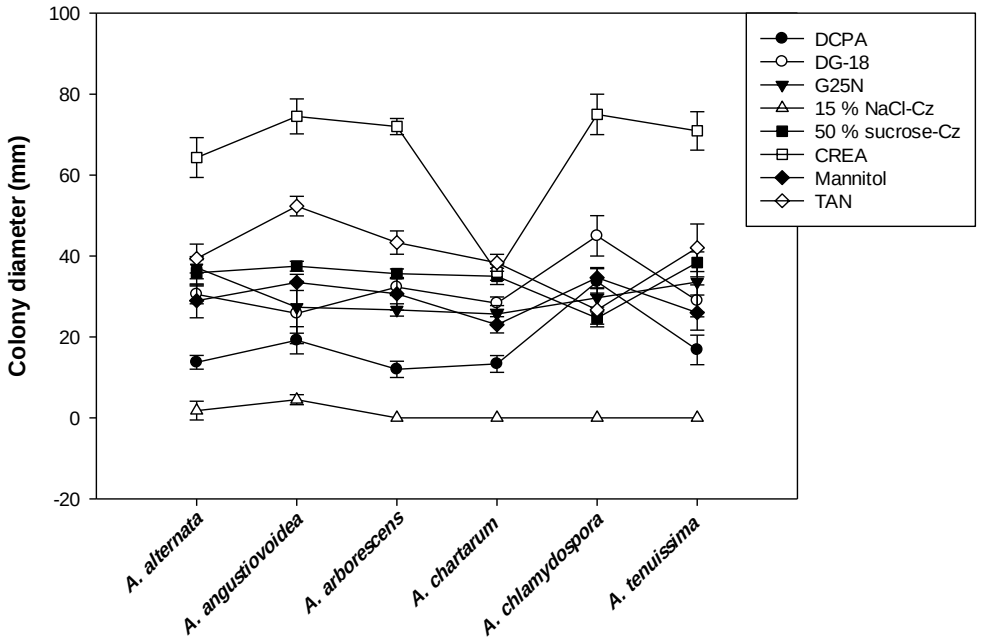

Figure 4: Effect of growth media on the growth of *Alternaria* species.

Table 5: Enzymes production by *Alternaria* species

***Alternaria* species**	**Peroxidase**	**Pyrocatechol oxidase**	**Urease**
A. alternata (N=5)	H, M	H, M	H, M
A. angustiovoidea (2)	W	H	H
A. arborescens (1)	M	H	H
A. chartarum (1)	M	H	H
A. chlamydospora (1)	-ve	-ve	M
A. tenuissima (5)	H, M	H, M	H, M

N= Number of isolates; H = high; M = moderate; W= weak; -ve= negative to test

4. DISCUSSION

In the present study, DG18 medium recorded the highest total count of 11236 CFU/g soil over the remaining isolation media, however, it ranked the last in the number of genera (26) and species (67).This may be attributed to its low water activity which is appropriate for the growth of only xerophilic and xerotolerant fungi [26]. The most common fungi obtained during the present study were *Aspergillus brasiliensis, A. flavus, A. niger, A.*

terreus, *Alternaria alternata*, *A. tenuissima*, *Curvularia lunata*, *C. spicifera*, *C. tsudae Fusarium chlamydosporum*, *F. oxysporum*, *F. solani* and *Penicillium chrysogenum.* These common fungi were also the predominant genera and species in several studies on the Egyptian soils [4, 6-11, 49-52]. On AWPDA, *Aspergillus* ranked the first followed by *Fusarium* and *Penicillium*. *Aspergillus niger*, *A. terreus*, and *Fusarium solani* were the prevalent species. These findings were unlike those obtained by Hong and Pryor [24] who reported *Alternaria* as the prevalent genus in their study accounting 63.6 %-81.0 % of recovered fungal isolates.

On DCPA, *Aspergillus flavus*, *A. niger*, *A. terreus*, *Fusarium solani* and *Penicillium chrysogenum* were the most common species. In this respect, Andrews and Pitt [25] isolated *Alternaria*, *Aspergillus*, *Chaetomium*, *Epicoccum*, *Fusarium*, and *Phoma* using DCPA medium, with *A. tenuissima*, *A. flavus*, *A. niger*, *F. graminearum*, *F. semitectum* were the most prevalent species.

On DG18, *Aspergillus* showed the highest total count and frequency and ranked the second. *Aspergillus niger*, *A. flavus*, *A. terreus*, *A. nidulans* were the highest frequent species. These results are in agreement with the findings reported by Hocking and Pitt [26] who used DG18 for isolation of xerophilic fungi from dried foods and they stated that *A. amstelodami* and *A. umbrosus* were recorded as the most frequent species.

On PCA-Mn, *Aspergillus* and *Fusarium* were the most predominant genera. The most abundant species were represented by *Aspergillus niger*, *A. flavus* and *Fusarium solani*. Also, Sørensen, Mogensen [27] used potato-carrot agar with manganese as a semi-selective medium for isolation of *Alternaria* spp., *Epicoccum* spp., and *Phoma* spp. from soil. Regarding the impact of carbon and nitrogen sources on growth and base production by *Alternaria* species, all the fifteen isolates could grow on ammonium tartarate, ammonium oxalate and glycine, while 14, 13 and 11 isolates could grow on citric acid, lactic acid and sodium nitrite, respectively. *A. angustiovoidea* and *A. tenuissima* were highly base producers on ammonium tartarate. The consumption of different carbon and nitrogen compounds based mainly on the enzymes activity produced by the fungus. Thaware, Fugro [53] tested the impact of different carbon sources on the growth of *A. alternata*. and they revealed that no growth was observed on citric acid.

The effect of different pH values on growth of *Alternaria* species showed that the maximum growth of *A. alternata*, *A. chlamydospora* and *A. tenuissima* was observed at pH 5.0, while pH 9.0 and pH 11.0 were the best for the growth of *A. angustiovoidea* and *A. chlamydospora*, respectively. The hydrogen ion concentration is a limiting factor for fungal growth due to its effect on preparing suitable solution for growth and metabolism [54]. Although, Kannan, Mohan [55] reported that *Alternaria* growth was reduced at the high pH levels. Extensive studies were conducted to determine the optimum pH values for growth of *A. alternata*, and they were stated that *Alternaria* species prefer weak acidity conditions for growth, where the peak of their growth was detected at pH 4.5 [56], pH 5.4 [57], pH 5.5 [58], or pH 6.5 [59].

The impact of different temperatures on growth of *Alternaria* species was estimated at 5, 35 and 45 °C. The results showed that all *Alternaria* isolates tested could grow at 5 ºC and 35 ºC, while no growth was recorded at 45 ºC. This may be attributed to that high temperature is considered as thermal death point for some *Alternaria* species [56]. The maximum growth of *A. alternata, A. angustiovoidea, A. chlamydospora* and *A. tenuissima* was observed at 35 ºC while *A. chartarum* at 5 ºC. In harmony with our results, *A. alternata* showed the best growth at 30 ºC [59] or 35 ºC [60]. But in other study, the critical temperature degree was found to vary among different *Alternaria* strains [56].

4.1. Description of the encountered species of *Alternaria*

Morphological features are considered as a fundamental tool in the identification and differentiation between several *Alternaria* species, although, they are not satisfactory in some cases. Conidial size, septation, beak length, in addition to, the number of conidia in the chain are the most considered characters (Figure 5).

1. *Alternaria alternata* AUMC 14161

a. Morphological characteristics

Macroscopic: Colony diameter exceeding 60 mm diam after 7 days on both PCA and V-8 at 22 °C, but slow growth on DCPA (9 mm). The isolates

tested showed three concentric rings and good sporulation on PCA and V-8, whereas, conidiogenesis is sparse on DCPA.

Microscopic: Conidiophore golden brown, verrucose in maturation, simple or geniculate, 40-60 × 3-5 µm, with an apical cluster of branching chain of 15-20 conidia. The first 1-2 conidia in the chain usually long elliptical, 25-48 × 5-15 µm with 4-7 transverse septa and 0-1 longitudinal septum. The latter conidia ovoid, subspheroid, 7-25 × 11-17 µm, commonly with 3-7 transverse septa and at least one longitudinal septum. Secondary conidiophore is sometimes produced, one-celled, 2-8 × 3-5 µm or 2-celled, up to 30 µm long.

Ramjegathesh and Ebenezar [56] described 10 isolates of *A. alternata* with muriform conidia and they reported that their conidia ranged from 31.0 to 42.5 µm long. Macro- and microscopic features of *A. alternata* isolates in this study were closely related to that described by Simmons [19] but with minor variation in conidial size.

b. Physiology

The five isolates of *A. alternata* tested could grow on all carbon and nitrogen sources. In addition, they were highly producers of pyrocatechol oxidase and urease enzymes.

2. *Alternaria angustiovoidea* AUMC 14107

a. Morphological characteristics

Macroscopic: Colony attaining diameter of 50-60 mm on both V-8 and PCA at 22 °C. On contrast, they showed slow growth on DCPA with 3 pairs of poorly-defined concentric rings of growth. Conidiogenesis is good on PCA, moderate on V-8 and no sporulation on DCPA.

Microscopic: Conidial chains, short, unbranched with 6-8 (-10) conidia. Juvenile conidia (0-1 transverse septum) 5-18 × 4-6 µm. Mature conidia are narrow-ovoid with 3-8 transverse septa and 0-1 longitudinal septum in each transverse segment, median septum is darker and more constricting than the other septa, 50-75 × 10-15 µm, have narrow-taper beaks or secondary conidiophore 50-75 µm, ornamented dilute to dull yellow brown. Other conidia are broad ovoid to ellipsoid and lack narrow beak extensions, 20-40 × 7-14 µm with 4-7 transverse septa and one longitudinal septum at the widest cell.

These characters are in harmony with the description of Simmons [19], although our isolates showed some variations in colony diameters. The slight difference in description between the local *Alternaria* isolates and those reported in the manual of Simmons [19] maybe due to variation in their environmental habitats. It is worth mentioning that, this species was recorded for the first time in Egypt from soil of Assiut Governorate, Egypt [61].

b. Physiology: The two isolates tested of *Alternaria angustiovoidea* tested could grow on all carbon sources, as well as on ammonium oxalate and glycine and on sodium nitrite similar to *A. alternata*. They were high producers of pyrocatechol oxidase and urease but moderate producer of peroxidase enzyme.

3. *Alternaria arborescens* AUMC 14106

a. Morphological characteristics

Macroscopic: Colony expanding to 50 mm diam after 7 days at 22 °C showing 3 concentric rings and good sporulation on both PCA and V-8 media.

Microscopic: Primary conidiophore long, bearing terminal cluster of branches and conidial chains, simple or aggregated to form funiculose robe, forming arborescent like structure. Conidiophores 500 (-800) × 4-5 µm. Conidial chain contains 1-5 conidia. Conidia punctate to verrucose, with dilute tan to brown color, 17-36 × 7-13 µm with 1-4 transverse septa and 1-2 longitudinal or oblique septa. Secondary conidiophores formed at the apical portion of terminal conidia, simple or geniculate with conidiogenous site at each bend, short, 4-6 × 3-4 µm.

b. Physiology: This species (one isolate) was failed to grow on either lactic acid (as a sole carbon source), sodium nitrite (as a sole nitrogen source), or at the three temperature degrees tested, but it is high producer of both pyrocatechol oxidase and urease.

The species was also recorded for the first time in Egypt from soil collected from Assiut Governorate, Egypt [61].

4. *Alternaria chartarum* AUMC 14162

a. Morphological characteristics

Macroscopic: Colony attaining 60 mm diameter on both V-8 and PCA at 22 °C after 7 days, slow growing on DCPA with 2 pairs of poorly-defined

concentric rings. Conidiogenesis on PCA and V-8 moderate and no sporulation on DCPA.

Microscopic: Conidiophores simple or branched, geniculate with multiple conidiogenous loci, up to 50 × 5-7 µm. Apical secondary conidiophores with multiple conidiogenous sites and lateral secondary conidiophores. Conidia ellipsoid or obovoid, beakless, with a narrow base, golden brown to dark brown, smooth to verrucose, with 1-4 transverse and one longitudinal or oblique septa in each segment, 15-35 × 7-15 µm.

b. Physiology: The isolate tested was able to grow on all carbon and nitrogen sources but weakly produce base. In addition, it was high producer of pyrocatechol oxidase and moderate producer of urease.

5. *Alternaria chlamydospora* AUMC 14163

a. Morphological characteristics

Macroscopic: Colony growing rapidly to 65-70 mm diam on PCA and V-8 after 7 days at 22 °C, but very slow growing on DCPA (10 mm). The isolates tested showed 2 well-defined pairs of concentric rings and good sporulation on V-8.

Microscopic: Colonies floccose, blackish-brown. Arachnoid to spiral hyphae are abundant on the agar surface. Conidiophores up to 150 × 3-6 µm, pale brown. Conidia formed in short chains of 5-8 µm and sometimes solitary. Conidia beakless, ovoid or subellipsoid, swelling and becoming variable in shape, ca 35-50 × 16-22 µm with 6 transverse septa and 1-2 longitudinal or oblique septa. Smaller conidia 12-20 × 8-13 µm with 1-2 transverse septa, smooth to finely verruculose, pale to golden brown, constricted at 3-4 transverse septa.

The description of our species is closely related to that described by Simmons [19], who, recorded the same morphological features with the exception of the aggregation of the arachnoid hyphae forming black swellings.

b. Physiology

The isolate tested was characterized by positive growth on ammonium tartarate, citric acid and lactic acid (as sole carbon sources) and

ammonium oxalate and glycine (as sole nitrogen sources). Besides, it was high base producer on lactic acid and moderate urease production.

6. *Alternaria tenuissima* AUMC 14164

a. Morphological characteristics

Macroscopic: Colonies rapidly growing attaining 50 mm and 55 mm diam after 7 days at 22 °C on PCA and V-8 respectively, slow growing on DCPA. The five isolates tested showed good sporulation on PCA and V-8, but no sporulation was observed on DCPA.

Microscopic: Conidial chain simple, 5-15 conidia, or may produce one or more lateral branches of a few conidia. First conidium in the chain has narrow tapered upper half, but apical conidia ovoid to ellipsoid without a narrow taper in the upper half, each conidium produces a short apical secondary conidiophore of 1-2 cells. Conidia golden brown, conspicuously punctate, with transverse septa only, 30-48 × 8-12 µm or with transverse and longitudinal septa, obclavate, 30-45 × 14-18 µm. Conidia constricted at the median transverse septum.

b. Physiology: The five isolates tested have variable rates of growth on both carbon and nitrogen sources, except one isolate failed to grow on both citric and lactic acids, and 2 isolates could not grow on sodium nitrite. The isolates tested showed high peroxidase, pyrocatechol oxidase and moderate urease production.

In the current study, some morphological features could be efficiently used to differentiate some *Alternaria* species. For example, chlamydospores and conidial width are good criteria to distinguish between *A. chlamydospora*, and *A. angustiovoidea*. Also, *A. alternata* and *A. arborescens* could be separated by conidiophore length and the growth capability on 15 % NaCl-Cz. The morphologically closely related *Alternaria* species or species with doubtful identification should be subjected to molecular identification, but in the developing countries, this technique is not constantly at the hand, and maybe available but high-coasted. For this reason, we studied some physiological and biochemical behaviors of the tested *Alternaria* isolates, willing to be used as diagnostic features. In this respect, base production on carbon and nitrogen sources, growth at different pH values and growth on 15 % NaCl-Cz are good diagnostic criteria.

The growth of fungi depends on the behavior of the fungal strain, environmental and nutritional conditions [62]. In the present investigation, all tested media were favorable for the growth of all *Alternaria* isolates tested, although, the maximum growth was observed in creatine-sucrose medium, followed by TAN, $G_{25}N$ agar. On the other hand, only 4 isolates of *A. alternata* and *A. angustiovoidea* (2 isolates each) were able to grow on 15 % NaCl-Cz. Growth requirements of fungi based on the substrate, which secure their food and energy, may be due to the variation in the nutritional requirement of the strain.

The current data revealed that, 9, 13 and 14 (out of 15 tested) *Alternaria* isolates were respectively able to produce, peroxidase, and pyrocatechol oxidase and urease enzymes with variable degrees. Moreover, other isolates belonging to *A. angustiovoidea* (2 isolates) showed maximum production of pyrocatechol oxidase and urease. In this respect, a strain of *A. alternata* tested by Sharma, Aggarwal [63] could produce peroxidase.

Dichotomous key for encountered *Alternaria* species

1. Conidia in chains (branched or unbranched)	**2**
- Conidia solitary, obovoid, with rounded tip (non-beaked), conidiophores conspicuously geniculate	***A. chartarum***
2. Conidia in unbranched chains	**3**
- Conidia in freely branched chains	**5**
3. Conidial chain short, 4-8 conidia	**4**
- Conidial chains long, 10-25 conidia, terminal conidia with a narrow apical extension	***A. tenuissima***
4. Chlamydospores present, conidia ovoid or ellipsoid, 16-22 µm width, intensive base production on lactic acid, no base production on glycine, rapid growth at pH 11 (up to 80 mm diam), negative growth on 15 % NaCl-Cz	***A. chlamydospora***
- Chlamydospores absent, conidia long, narrow-ellipsoid, 10-15 µm width, intensive base production on glycine, no base production on lactic acid, positive growth on 15 % NaCl-Cz and intensive base production on glycine	***A. angustiovoidea***
5. Conidia in long branched and clumps chains near a short primary conidiophore apex (up to 200 µm long), moderate growth on 15 % NaCl-Cz	***A. alternata***
- Conidia aggregated in open branched tufts near the apex of long conidiophore (up to 1 mm long), no growth on 15 % NaCl-Cz	***A. arborescens***

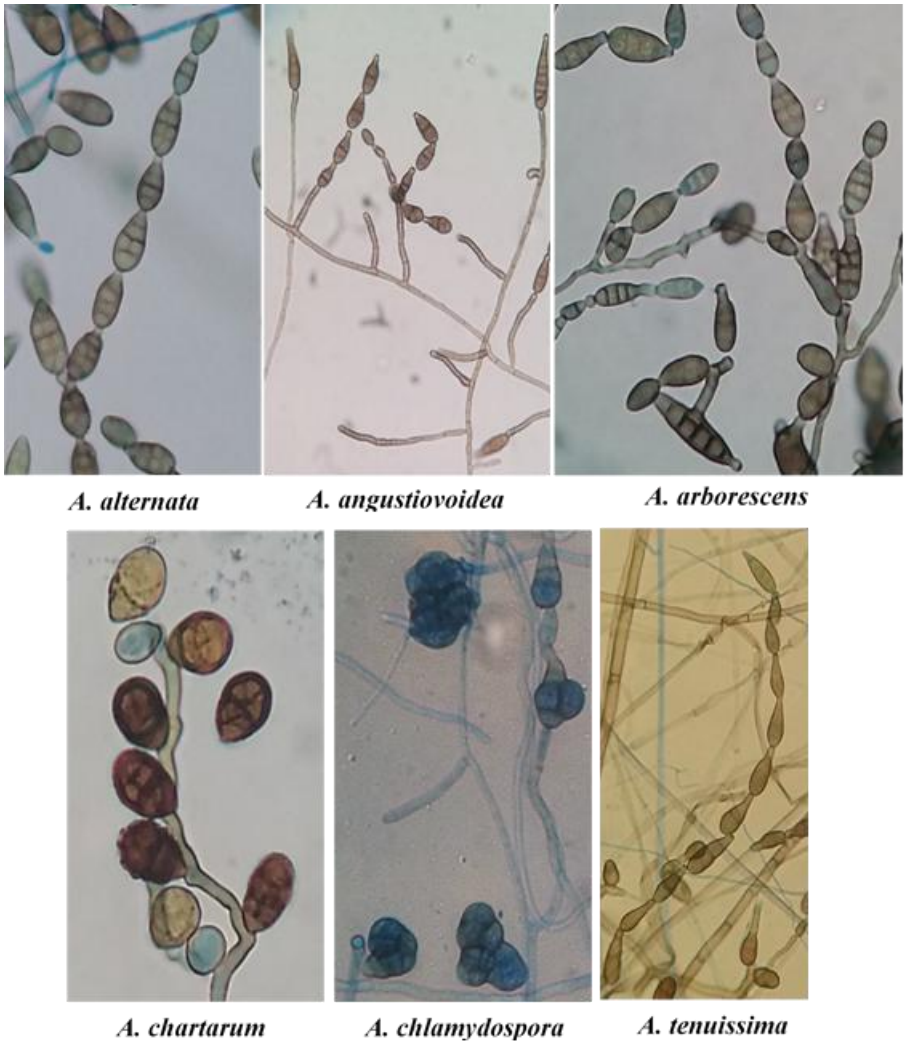

Figure 5: Microscopic characteristics (conidia and conidiophores) of *Alternaria* species encountered in the present study.

CONCLUSION

AWPDA is the richest medium in species diversity (81 species), although DG18 showed the maximum total count (1123600 CFUs). *Aspergillus*, *Fusarium* and *Penicillium* represented the most common genera, whereas the most predominant species were *A. niger*, *A. terreus*, *A. flavus* and *F. solani*. AWPDA and PCA-Mn media harbored 5 out of 6 *Alternaria* species. Based on morphological features and physiological

aspects, *Alternaria alternata* differs from *A. arborescens* by short primary conidiophores and positive- growth on 15 % NaCl-Cz, *A. chlamydospora* is distinguished from other species by chlamydospore-like conidia and high base production on lactic acid. *A. chartarum* is characterized by solitary conidia and conspicuously geniculate conidiophores. Moreover, *A. tenuissima* was distinguished from *A. angustiovoidea* by long conidial chains of up to 20 conidia and conidial width. Our results concluded that, some physiological features are of valuable criteria that may be aid in confirmation of the identification of doubtful or miss-identified isolates if DNA sequencing is unavailable.

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تقييم الخصائص الفسيولوجية والبيوكيميائية لأنواع جنس الألترناريا المعزولة من محافظة أسيوط ، مصر بالإضافة الى مفتاح لتعريف الأنواع المعزولة

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إستهدفت الدراسة الحالية عزل وتعريف الأجناس والأنواع الفطرية الموجودة فى عينات من التربة تم تجميعها من مزرعة قسم النبات والميكروبيولوجى بكلية العلوم، جامعة أسيوط على أربعة أوساط غذائية وهى AWPDA ، DCPA، DG-18 و PCA-Mn عند درجة حرارة ٢٥ مئوية.

تم عزل وتعريف مائة وإثنان وعشرون نوعا فطرياً تنتمى إلى سبعة وأربعين جنساً، وقد كان جنس *Aspergillus* هو الأكثر إنتشاراً على الأربعة أوساط غذائية، تبعه جنس *Fusarium* ثم *Alternaria*، *Curvularia*، *Talaromyces*، *Penicillium* و *Stachybotrys*. وقد كان *Aspergillus niger* هو أكثر الأنواع سيادة على كلا من AWPDA، DG-18 و PCA-Mn.

وقد إستهدفت الدراسة الحالية أيضاً ولأول مرة فى مصر تقييم بعض الإختبارات الفسيولوجية و البيوكيميائية لستة أنواع من *Alternaria* ممثلة بخمسة عشر معزولة، وقد تم إجراء تحليل إحصائى للنتائج وكانت النتائج كما يلى:

١- إستطاعت ١٤، ١٣، ١١ معزولة النمو على حمض الستريك وحمض اللاكتيك ونيتريت الصوديوم على التوالى.

٢- كانت *Alternaria tenuissima* و *A. angustiovoidea* هما الأكثر إنتاجاً للقلويات عند نموهما على وسط غذائى يحتوى على ترترات الأمونيوم كمصدر وحيد للكربون، بينما كانت *A. angustiovoidea* و *A. arborescens* هما الأكثر انتاجاً للقلويات على وسط الجليسين.

٣- إستطاعت كل المعزولات أن تنمو على وسط creatine-sucrose وكانت *A. arborescens* هى أقوى العزلات مقدرة على تغير لون الوسط بينما تراوحت باقى العزلات من السلبى إلى المتوسط.

٤- أظهرت *A. chlamydospora* أكبر نمو عل وسط mannitol و *A. angustiovoidea* على وسط tannin-sucrose.

٥- كان إختبار النمو على وسط NaCl-Cz 15 % مميز لنمو ٦٠ % من عزلات *A. alternata* و ٥٠ % من عزلات *A. angustiovoidea*.