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Phyllosphere and Phylloplane Fungi of Banana Cultivated in Upper Egypt and their Cellulolytic Ability

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Seventy-three species and five varieties belonging to 36 genera were collected from leaf surfaces of banana plants on glucose and cellulose-Czapek's agar at 28°C. The results obtained from leaf surfaces (phyllosphere and phylloplane) were basically similar on the two types of media and the most common fungi were *Alternaria, Aspergillus, Chaetomium, Cladosporium, Cochliobolus, Curvularia, Gibberella, Memnoniella, Mycosphaerella, Setosphaeria* and *Stachybotrys*. The monthly counts of these fungi were irregularly fluctuated giving maxima at various months. *Chaetomium globosum* was in the top of fungi in producing both exo- and endo-β-1,4-glucanases among the 34 tested isolates obtained from leaves (phylloplane) on cellulose-Czapek's agar. Maximum production of these enzymes by *C. globosum* was 6 and 8 days after incubation at 25°C with culture medium containing wheat bran as a carbon source and peptone as a nitrogen source and initially adjusted to pH 6.

KEYWORDS: Banana, Cellulolytic ability, Phyllosphere fungi, Phylloplane fungi

Numerous investigations have been carried out on the fungus flora of leaf surfaces of several plants growing or cultivated in many parts of the world by several researchers (Abdel-Fattah *et al.*, 1977; Abdel-Hafez, 1981, 1984, 1985; Abdel-Hafez *et al.*, 1995; Eicker, 1976; Khallil and Abdel-Sater, 1993; Mazen *et al.*, 1985; Nagaraja, 1991; Perez and Mauri, 1989; Sharma, 1974).

Banana is one of the most important crops cultivated in Upper Egypt. Little is known about the mycoflora of banana, hence any information on the mycoflora of banana is very important. The aim of the present investigation is to study the fungal flora of leaf surfaces, cellulolytic activity of some fungal isolates and the effect of some environmental and nutritional factors on cellulase production.

Materials and Methods

Samples of banana leaves were collected from banana fields in Qena Governorate every fortinight during the peroid January-December 1999. Samples were placed in polyethylene bags and transferred immediately to the laboratory for isolation of various groups of fungi on 1% glucose and 1% cellulose-Czapek's agar.

Determination of phyllosphere fungi. The dilution plate method was used as employed by Abdel-Hafez (1985). A known weight of banana leaves segment were washed with a known volume of distilled water to obtain the desired final dilution. One ml of inal dilution was transferred to a steril petridish and poured with melted, but colled agar medium.

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Determination of phylloplane fungi. Banana leaves were subjected to a series of washing with sterile distrilled water. Thereafter, they were throughly dried between sterile filter papers. Four segments were placed on the surface of the agar medium in each plate (Abdel-Fatth *et al.*, 1977).

Screening of fungal isolates for cellulase production. Thirty-four fungal isolates representing to 23 genera were screened for their abilities to produce exo- and endo-β-1,4-glucanase (C_1 and Cx enzymes, respectively). Isolates were cultured on Eggins and Pugh medium (1962) and pH was adjusted to 5.4 using acetate buffer. Cultures were incubated at 28°C for 7 days. Using a sterile cork borer, 10 min diameter, discs were cut to inoculate 50 ml sterile liquid medium (in 250 ml Erlenmeyer conical flasks) of Eggins and Pugh medium (1962) for exo-β-1,4-glucanase production and Prasad and Verma medium (1979) for endo-β-1,4-glucanase. After 7 days incubation at 28°C the cultures were filtered and the filtrates were used to detect the activity of enzymes.

Detection of exo- and endo-β-1,4-glucanase. Using a sterile cork borer three cavities (10 min diameter) were made in plates containing solid Eggins and Pugh medium (1962) and solid medium of Dingle *et al.* (1953) for detection both exo- and endo-β-1,4-glucanase, respectively. 0.1 ml of culture filtrate was dropped in each of these cavities followed by incubation at 28°C for 24 hours, then the plates were flooded with chloroiodide of zinc solution and the uncoloured zone gave a measure of cellulolytic power of isolates.

Factors affecting cellulase production. The effect of different ecological and nutritional factors on production

of cellulases by *Chaetomium globosum* was studied, since this species was found to be highly active in cellulases production.

Effect of temperature and time course. The inoculated flasks were incubated at 20, 25, 30, and 40°C for 14 days and harvested at 48h intervals. Culture fluids were filtered and centrifugated at 5000 rpm. for 10 min. The clear supernatents were assayed for enzymes activity.

Effect of pH values. The test isolate was grown on the basal medium of Deacon (1985). The initial medium was adjusted with 0.1 N NaOH or 0.1 N HCl to different values of pH ranging from 2 to 12. After inoculation, cultures were incubated at 25°C for 6 days for C_1 and 8 for Cx. At the end of incubation period the cultures were filtered, centrifugated and the clear supernatents were assayed for cellulase activity.

Effect of different carbon sources. The basal medium (Deacon 1985) with pH 6 (the best pH for cellulases production) was supplemented with 1% of one of the following carbon sources: CMC, filter paper, cellulose powder, wheat bran, wheat straw, maltose and clover straw. The inoculated flasks were incubated at 25°C for 6 or 8 days (the best incubation periods for C_1 and Cx enzymes, respectively) and the cultures were filtered. After centrifugation the filtrate was used to detect the cellulase activity.

Effect of different nitrogen sources. Sodium nitrate (2 g/l) in the basal medium were replaced by the same amount of various nitrogen compounds such as $NaNO_2$, KNO_3, $(NH_4)_2SO_4$, yeast extract and peptone in addition to $NaNO_3$ as a control. Cultures in flasks were incubated at 25°C for 6 or 8 days and the cultures were filtered, centrifugated and the filtrate was used for the detection of cellulase activity. Assay for cellulase activity, the method described by Naguib (1964) was employed.

Results and Discussion

The monthly total counts of phyllosphere and phylloplane surfaces fungi of banana on plates of glucose and cellulose-Czapek's agar irregularly fluctuated giving peaks during February and January, respectively.

Seventy-three species and five varieties belonging to 36 genera were collected from phyllosphere (35 genera and 66 species +4 varieties) and phylloplane (30 and 48+2 var.) of banana leaves on glucose and cellulose-Czapk's agar at 28°C (Table 1). The most common fungi from the two substrates on the two types of media were: *Alternaria alternata*, *A. tenuissima*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Cochliobolus lunatus*, *C. spicifer*, *Gibberella fujikuroi*, *Mycosphaerella tassiana*, *Setosphaeria rostrata* and *Stachybotrys chartarum*. Also, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Memnoniella subsimplex* and *Myrothecium verrucaria* were prevalent in

Table 1. Total counts of phyllosphere (per g fresh leaf) and phylloplane (480 segments) fungi, number of cases of isolation and occurrence remarks on glucose and cellulose-Czapek's agar at 28°C

Genera and species	Phyllosphere				Phylloplane			
	Glucose		Cellulose		Glucose		Cellulose	
	TC[a]	NCI&OR	TC	NCI&OR	TC	NCI&OR	TC	NCI&OR
Acremonium strictum W. Gams	1900	3L	1850	3L	13	2R	16	3L
Alternaria	6900	20H	7200	19H	101	23H	116	17H
A. alternata (Fries) Keissler	6050	20H	6400	19H	69	18H	95	16H
A. citri Ellis & Pierce	–	–	–	–	5	1R	–	–
A. raphani Grosves Skolko	350	5L	300	5L	8	3L	7	2R
A. tenuissima (Kunze:Pers.) Wiltshire	500	6M	500	5L	19	8M	14	8M
Aspergillus	19500	16H	6800	14H	18	12H	–	–
A. candidus Link	50	2R	–	–	–	–	–	–
A. flavus Link	3250	12H	1200	12H	10	12H	–	–
A. fumigatus Fresenius	4850	14H	1950	13H	1	1R	–	–
A. niger Van Tieghem	9300	12H	2950	14H	7	3L	–	–
A. ochraceus Wilhelm	150	3L	100	2R	–	–	–	–
A. terreus Thom	1200	6M	450	5L	–	–	–	–
A. terreus var. *aureus* Thom & Raper	550	5L	–	–	–	–	–	–
A. ustus Fennell & Raper	50	1R	150	2R	–	–	–	–
A. versicolor (vuill.) Tiraboschi	100	2R	–	–	–	–	–	–
Botryotrichum atrogriseum Van Beyma	100	2R	350	2R	–	–	–	–
Chaetomium globosum Kunze	600	12H	600	12H	10	6M	41	12H
Circinella muscae (soroke.) Berl. & Detoni	500	5L	–	–	–	–	–	–

Table 1. Continued

Genera and species	Phyllosphere				Phylloplane			
	Glucose		Cellulose		Glucose		Cellulose	
Cladosporium	11150	15H	11000	16H	28	10M	44	12H
C. cladosporioides (Fres.) de Vries	9550	12H	11000	16H	28	10M	35	8M
C. sphaerospermum Penzig	1600	10M	–	–	–	–	9	5L
Cochliobolus	2750	14H	1000	12H	63	12H	63	12H
C. bicolor Paul & Parbery	150	1R	–	–	–	–	–	–
C. hawaiienesis Alcorn, Trans	–	–	–	–	5	1R	–	–
C. intermedius Nelson	250	1R	–	–	–	–	–	–
C. lunatus Nelson & Haasis	1400	14H	950	12H	29	12H	35	12H
C. spicifer Nelson	950	12H	50	1R	29	12H	28	12H
Cunninghamella echinulata Thaxter	–	–	150	2R	–	–	–	–
Curvularia	1300	12H	1400	13H	18	8L	42	12H
C. clavata Jain	200	4L	50	1R	7	5L	9	4L
C. lunata var. *aeria* (Batista, Lima & Vasconcelos)	350	5L	600	5L	–	–	18	5L
C. ovoidea (Hiroe & Watan.) Muntanola	150	2R	150	2R	5	1R	–	–
C. pallescens Boedija	600	5L	600	4L	6	2R	15	3L
Cunninghamella echinulata Thaxter	–	–	150	2R	–	–	–	–
Drechslera	600	6M	–	–	6	1R	–	–
D. neergaardii Danquah	–	–	–	–	6	1R	–	–
D. panendorforii (Van der Aa)	600	6M	–	–	–	–	–	–
Emericella	350	3L	150	2R	–	–	–	–
E. nidulans (Edidam) Vuillemin	50	1R	50	1R	–	–	–	–
E. nidulans var. *dentata* Sandhu & Sandhu	250	3L	50	1R	–	–	–	–
E. nidulans var. *lata* (Thom & raper) Subram.	–	–	50	1R	–	–	–	–
E. rugulosa (Thom & raper) Benjamin	50	1R	–	–	–	–	–	–
Epicoccum nigrum Link	100	2R	–	–	12	2R	–	–
Fusarium	450	6M	150	3L	16	3L	11	3L
F. nivale (Fr.) Ces.	200	4L	–	–	–	–	–	–
F. oxysporum Sheleecht.	250	3L	150	3L	16	3L	11	3L
Gibberella	550	11M	1000	12H	19	5L	30	12H
G. acuminata Wollenweber	–	–	400	4L	–	–	7	5L
G. fujikuroi (Sawada) Ito	550	11M	600	8M	13	2R	18	8M
G. intricans Wollenweber	–	–	–	–	6	1R	–	–
G. zeae (Schwabe) Petch	–	–	–	–	–	–	5	1R
Humicola grisea Traaen	–	–	–	–	–	–	7	1R
Khuskia oryzae Hudson	–	–	150	1R	–	–	4	1R
Macrophomina phaseolina (Tassi) Goid	50	1R	–	–	–	–	–	–
Memnoniella	3850	12H	4900	12H	3	3L	32	6M
M. echinata (Riv.) Galloway	1900	6M	150	3L	3	3L	9	5L
M. subsimplex (Cooke) Deighton	1950	11M	4750	10M	–	–	23	6M
Mucor	650	3L	–	–	13	3L	–	–
M. circinelloides Van Tieghem	500	3L	–	–	8	2R	–	–
M. hiemalis Wehmer	150	2R	–	–	5	2R	–	–
Mycosphaerella tassiana (Albertini & Schweinitz) Ditmer ex Steudel	10650	14H	5800	13H	19	11M	29	12H
Myrothecium	1250	5L	1550	6M	–	–	16	3L
M. roridum Tode	100	2R	–	–	–	–	–	–
M. verrucaria (Alb. & Sch.) Dit.	1150	3L	1550	7M	–	–	16	3L
Nectria haematococca Berkeley & Brown	150	3L	450	4L	24	11M	13	5L
Nigrospora sphaerica (Sacc.) Mason	250	3L	200	2R	3	1R	4	1R
Paecilomyces	1600	3L	1550	3L	–	–	13	2R
P. terricola (Miller, Giddens & Foster) Onions & Barron	–	–	1250	3L	–	–	13	2R

Table 1. Continued

Genera and species	Phyllosphere				Phylloplane			
	Glucose		Cellulose		Glucose		Cellulose	
P. variotii Bainier	1600	3L	300	1R	–	–	–	–
Penicillium	5350	15H	900	5L	3	1R	–	–
P. albdium Sopp	100	1R	-	-	–	–	–	–
P. chrysogemum Thom	2050	11M	200	3L	3	1R	–	–
P. citrimum Thom	1150	6M	–	–	–	–	–	–
P. corylophilum Dierckx	650	8M	150	2R	–	–	–	–
P. duclauxi Delacroix	250	3L	50	1R	–	–	–	–
P. funiculosum Thom	100	2R	100	2R	–	–	–	–
P. puberulum Bainier	1050	6M	400	3L	–	–	–	–
Phoma glomerata (Corda) Woolenweber & Hochapfel	–	–	50	1R	–	–	–	–
Rhizopus stolonifer (Ehrenb.) Lind	200	3L	–	–	11	3L	–	–
Scopulariopsis brevicaulis (Sacc.) Bainier	-	-	150	2R	2	1R	–	–
Setosphaeria rostrata Leonard	750	12H	300	6M	25	12H	36	12H
Stachybotrys	1200	12H	4050	13H	6	6M	24	12H
S. atra var. *microspora* Mathur & Sankhla	–	–	–	–	2	2R	–	–
S. chartarum (Ehrenb.:Lindt) Hughes	1200	12H	4050	13H	4	4L	24	12H
Stemphylium botryosum Wallroth	–	–	–	–	7	2L	22	5L
Torula herbarum (Pers.) Link	150	2R	100	2R	–	–	4	2R
Trichoderma	300	3L	350	3L	29	3L	28	5L
T. hamatum (Bonord.) Bain	50	1R	50	1R	9	1R	11	3L
T. pseudokoningii Rafai	250	3L	–	–	–	–	17	3L
T. viride Pers	–	–	300	3L	20	3L	–	–
Trichothecium roseum (Pers.) Link:Gary	50	1R	–	–	–	–	–	–
Ulocladium	400	3L	1650	5L	1	1R	30	5L
U. botrytis Preuss	–	–	1650	5L	1	1R	12	2R
U. tuberculatum Simmons	400	3L	–	–	–	–	18	3L
Verticillium lateritium Berkeley	500	3L	600	3L	–	–	7	1R
Total counts	74100		54450		70		632	
Number of genera = 36	35				30			
Number of species = 73 + 5 var.	66+4 var.				48+2 var.			

[a]TC = Total count in all samples; NCl = Number of cases of isolation (out of 24); OR = Occurrence remarks: H = high occurrence from 12-24 cases, M = moderate occurrence from 6-11 cases, L = low occurrence from 3-5 cases, R = rare occurrence 1 or 2 cases.

the two substrates on the suitable media. The monthly counts of the above fungal species were widely varied and fluctuated irregularly giving maxima during different months (Figs. 1 and 2). Also, some fungal species were common only in one or two substrates on one types of medium such as; in phyllosphere: *Aspergillus terrens, Cladosporium sphaerospermum, Drechslera panendorforfii, Memnoniella echinata, Penicillium chrysogemum, P. corylophilum* and *P. puberulum* on glucose and in phylloplane, and *Nectria haematococca* on glucose agar. Abdel-Hafez *et al.* (1995) isolated *Alternaria alternata, Aspergillus flavus, A. funigatus, A. niger, Cochliobolus lunatus, C. spicifer, Gibberella fujikuroi, Mycosphaerella tassiana, Penicillium chrysogemum* and *Setosphaeria rostrata* from leaf surfaces of sugarcane plant. All fungal species recovered from leaf surfaces of banana on the two types of media were previously isolated, but with different numbers and incidences from leaf surfaces of several plants growing or cultivated in many parts of the world (Abdel-Fattah *et al.*, 1977; Abdel-Hafez, 1981, 1984, 1985; Abdel-Hafez *et al.*, 1990, 1995; Abdel-Kader *et al.*, 1984; Abdel-Sater *et al.*, 1993; Collins, 1982; Lindsey and Pugh, 1976; Mazen *et al.*, 1985; Moubasher *et al.*, 1984; Stott, 1971; Vardavakis, 1988).

Cellulolytic activity of some fungal isolates. All fungal isolates screened for their abilities to produce C_1 and Cx enzymes on solid media proved to be active to utilize cellulose, but with different degrees (Table 2). Eight isolates (23.5% of total isolates) showed high cellulolytic activity in both exo- and endo-β-1,4-glucanase and these were *Acremonium strictum, Chaetomium globosum, Gibberella fujikuroi, G. zeae, Nectria haematococca, Setosphaeria rostrata, Stachybotrys chartarum* and *Trichoderma pseudokoningii*. Twelve isolates (35.3% of total isolates) showed high activity in production of C_1 enzyme

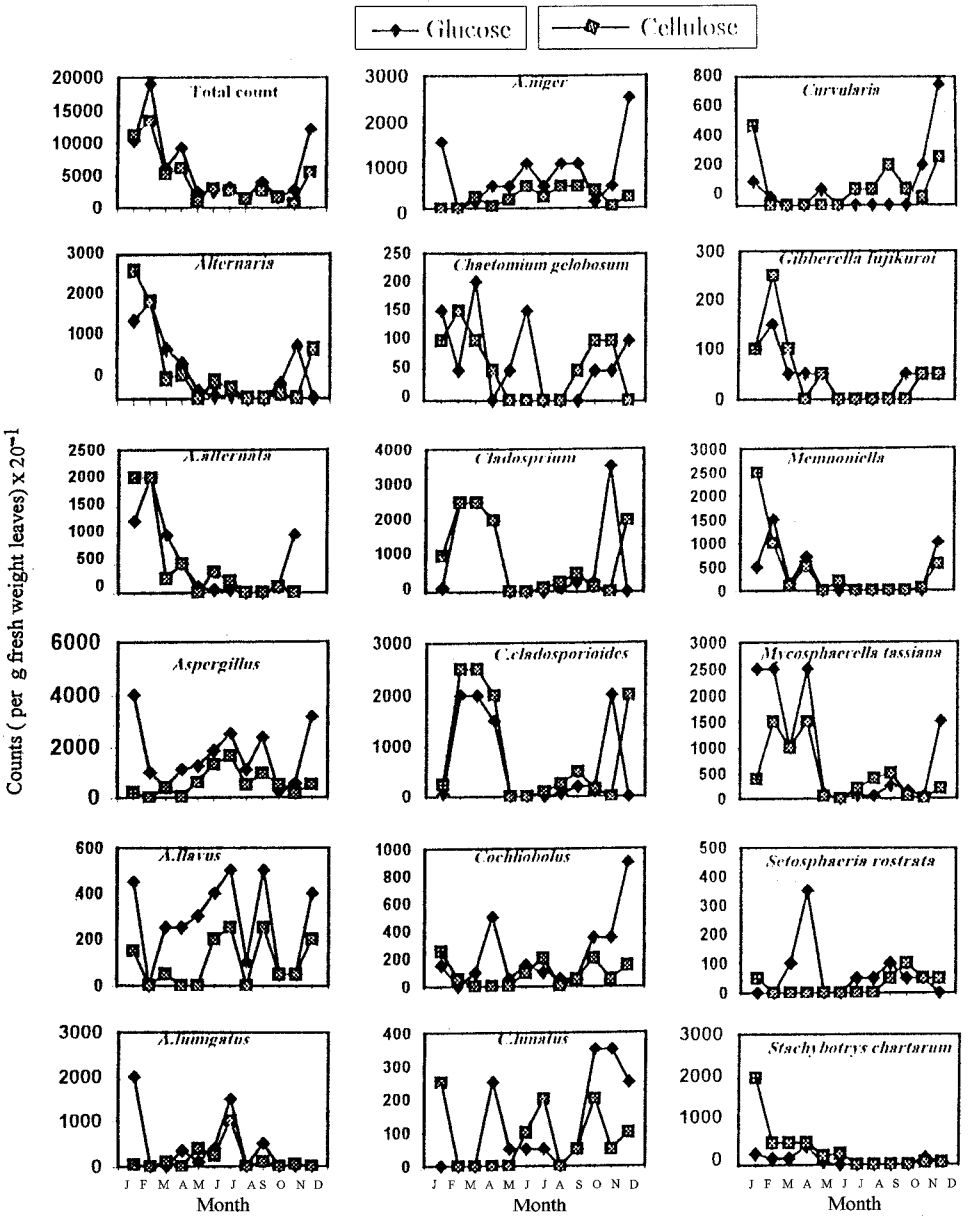

Fig. 1. Monthly counts (per g fresh leaves) of common phyllosphere fungi of banana plant on glucose and cellulose-Czapek's agar at 28°C.

only and these were *Alternaria raphani*, *Cladosporium cladosporioides*, *Cochliobolus lunatus*, *C. spicifer*, *Curvularia pallescens*, *Khuskia oryzae*, *Memnoniella subsimplex*, *Myrothecium verrucaria*, *Nigrospora sphaerica*, *Torula herbarum*, *Trichoderma hamatum* and *Ulocladium tuberculatum*. On the other hand, two isolates exhibited high activity production of Cx enzyme only and these were *Alternaria alternata* and *Fusarium oxysporium*. Six and 11 isolates (17.6% and 32.4% of total isolates) were found to be of moderate production of C_1 and Cx enzymes, respectively, while 8 and 13 isolates (23.5% and 38.2%) were of weak cellulolytic activity. Most of the above fungal isolates were reported as cellulase producers, but with variable capabilities by several workers (Abdal-Hafez *et al.*, 1995; Abraha and Gashe, 1992; Dubeauet *et al.*, 1986; Moharram *et al.*, 1993; Stewart *et al.*, 1983).

Chaetomium globosum was in the top of fungi in producing both two enzymes in this investigation. Maximum production of exo- and endo-β-1,4-glucanase by *C. globosum* was 6 and 8 days after incubation at 25°C with culture medium containing wheat bran as a carbon source and peptone as nitrogen source and initially adjusted to pH 6 (Figs. 3 and 4).

These findings are almost in agreement with those reported by Sandhu and Kalra (1985) who noticed that the maximum production of C_1 and Cx enzymes with *T. longibrachiatum* was achieved after 5 or 6 days of incubation

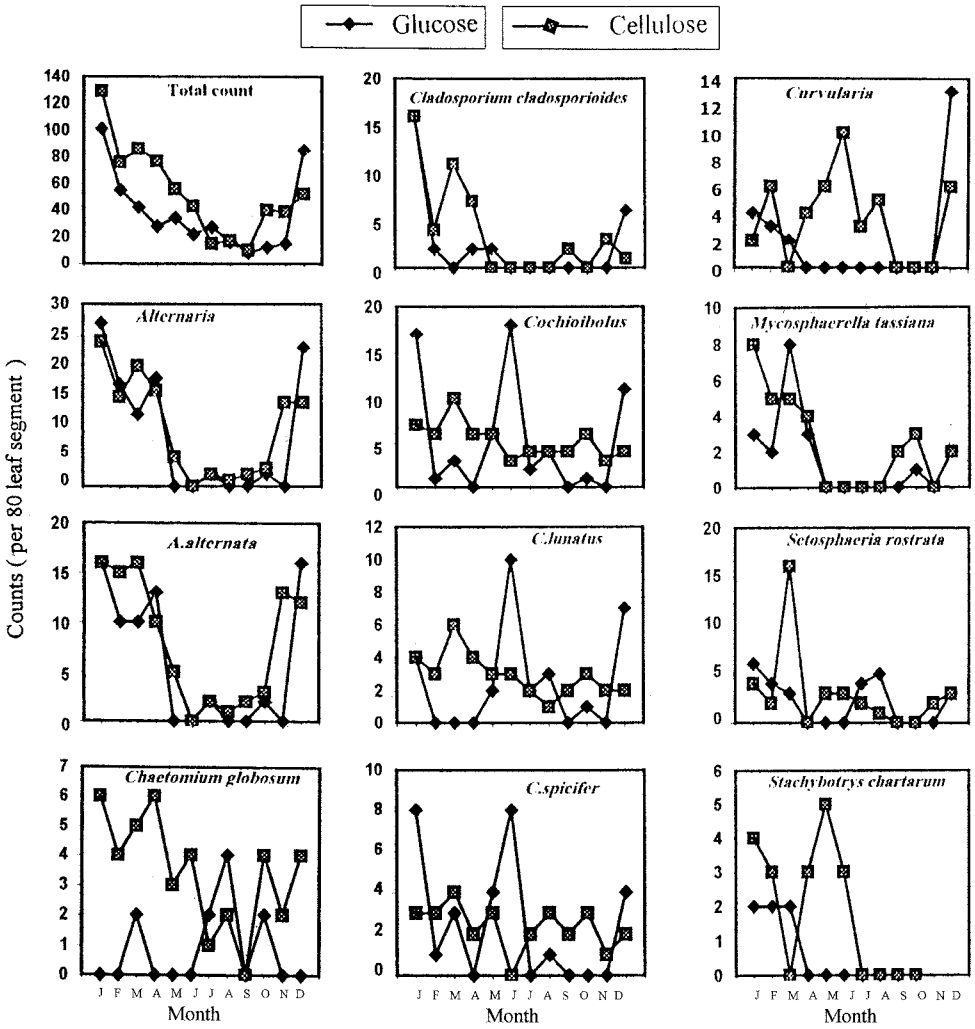

Fig. 2. Monthly counts (per 80 leaf segments) of common phylloplane fungi of banana plant on glucose and cellulose-Czapek's agar at 28°C.

Table 2. Degree of cellulolytic activies (calualated as diameter of clear zone in mm) of the fungal isolates tested

Fungal isolates	Exo-β-1,4-glucanase	Endo-β-1,4-glucanase
Acremonium strictum	21H[a]	20H
Alternaria alternata	19M	21H
A. raphani	20H	19M
A. tenuissima	13W	12W
Chaetomium globosum	26H	24H
Cladosporium cladosprioides	20H	17M
C. sphaerospermum	12W	11W
Cochliobolus lunatus	20H	16M
C. spicifer	20H	18M
Curvularia clavata	11W	14W
C. lunata var. *aeria*	19M	17M
C. pallescens	20H	18H
Fusarium oxysporum	18M	21H
Gibberella acuminata	14W	15W
G. fujikuroi	22H	20H
G. zeae	21H	20H
Humicola grisea	15W	13W
Khuskia oryzae	20H	13W

Table 2. Continued

Fungal isolates	Exo-β-1,4-glucanase	Endo-β-1,4-glucanase
Memnoniella echinata	16M	13W
M. subsimplex	20H	18M
Mycosphaerella tassiana	12W	11W
Myrothecium verrucaria	20H	13W
Nectria haematococca	24H	21H
Nigrospora sphaerica	20H	16M
Paecilomyces terricola	17M	15W
Setosphaeria rostrata	22H	20H
Stachybotrys chartarum	23H	21H
Stemphylium botryosum	14W	15W
Torula herbarum	20H	15W
Trichoderma hamatum	22H	19M
T. pseudokoningii	23H	22H
Ulocladium botrytis	18M	19M
U. tuberculatum	20H	17M
Verticillium lateritium	11W	12W

[a]Activity remarks: H = high, 20-28 mm; M = moderate, 16-19 mm; W = weak, 11-15 mm.

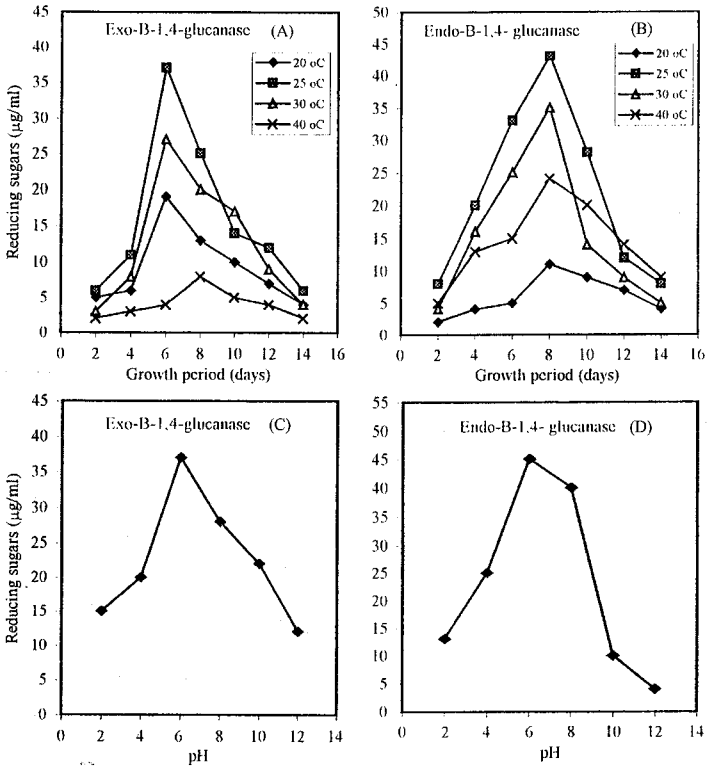

Fig. 3. Effect of time course and temperatures (A, B) and pH values (C, D) on production of exo- and endo-β-1,4-glucanse by *Chaetomium globosum*.

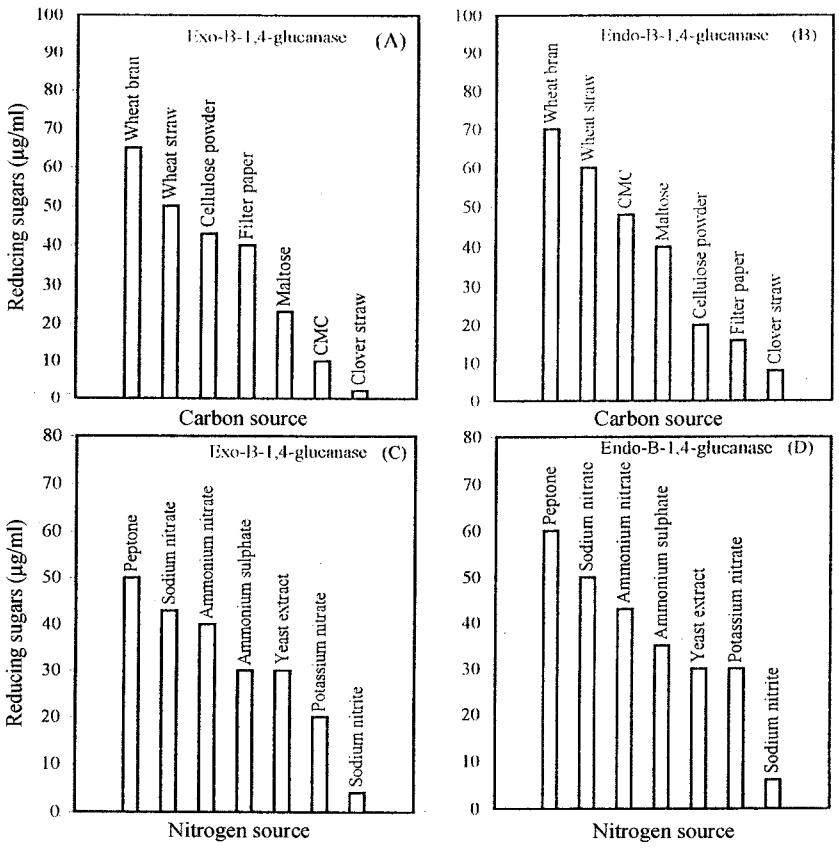

Fig. 4. Effect of different carbon (A, B) and nitrogen (C, D) sources on production of exo- and endo-β-1,4-glucanse by *Chaetomium globosum*.

at 27°C but with the incorportation of 1% lactose in culture medium which initially adjusted to pH 5. They, also found that CMC and malt extract were favourable for the enzymes production. Kalra and Sandhu (1986) found that the optimum pH temperature for cellulases in culture filterate of *T. harziamum* were 5~7 and 27°C, repectively. Nelly (1991) found that microcrystalline cellulose and pH 4.5 were the best coditions for production of C_1 and Cx enzymes by *T. reesei*. Also, Abdel-Hafez *et al.* (1995) found that the production of exo- and endo-β-1,4-glucanase by *T. viride* was 6 and 8 days after incubation at 25°C with culture medium containing wheat bran as a carbon source and pepton as nitrogen source and initially adjusted to pH 6.

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