

## EVALUATION OF METHODS FOR SCREENING OF EXTRACELLULAR CELLULASE IN FILAMENTOUS FUNGI

M.A. KHIYAMI\*\* and A.H. BAHKALI\*

\*Botany and Microbiology Department, College of Science, King Saud University, P. O. Box 2455, Riyadh 11451, Saudi Arabia.

\*\*King Abdulaziz City for Science and Technology, General Directorate of Research Grants, Riyadh, Saudi Arabia.

### تقييم طرق مسح نشاط أنزيم السيلوليز خارج الخلوي في بعض الفطريات الخيطية

محمد أحمد خيمي (١) و علي حسن عبد الرحمن بهكلي (٢)

(١) قسم النبات والأحياء الدقيقة - كلية العلوم - جامعة الملك سعود

ص . ب ٢٤٥٥

(٢) مدينة الملك عبد العزيز للعلوم والتقنية، الإدارة العامة لبرامج المنح

ص . ب ٦٠٨٦ الرياض ١١٤٤٢ ، المملكة العربية السعودية

#### الخلاصة

من الواضح أن العديد من الكائنات الحية الدقيقة قادرة على إنتاج الأنزيمات السيلولوزية خارج الخلية. ومن أهمها الفطريات الخيطية المحللة للمخلفات السيلولوزية بفعالية أكبر وذلك من خلال معدلاتها الأيضية النشطة. وتعتبر الأنواع التابعة لجنس ترايكوديرما *Trichoderma* من أفضل هذه الكائنات الخيطية. وتناولت الكثير من الأبحاث الأنظمة الأنزيمية السيلولوزية حيث ركزت دراستها على الأنواع التابعة لهذا الجنس.

تهدف هذه الدراسة إلى استخدام الثلاث طرق المختلفة الخاصة للمسح السريع للكشف عن الأنشطة الأنزيمية السيلولوزية للفطريات وهذه الطرق هي :

فحص انتشار الآجار *Agar diffusion test* ، تقانة المنطقة الشفافة *Cleared - zone Technique* وطريقة أزور السليلوز *Cellulose - azure Method* وأجريت الدراسة على ثمان وعشرين نوعا تابعا لخمسة عشر جنسا من أجناس الفطريات الخيطية من ترب مختلفة في المملكة العربية السعودية.

**Key words:** Saudi Arabia, Mycoflora Extracellular Cellulase, Screening.

#### ABSTRACT

Twenty-eight fungal species belonging to fifteen genera were isolated from Saudi Arabian soil and were screened for the production of extracellular cellulase enzymes. Cleared-zone technique, cellulose azure method and agar diffusion test for rapid screening were used. The cleared-zone technique was found to be much less reliable as compared to other methods. *Trichoderma viride*, *T. pseudokoningii* and *Aspergillus fumigatus* were the best producers of cellulase enzyme. The data obtained also suggest that caution is needed using these methods. The disadvantages of using one method for screening, which has been a common practise up to now, are discussed.

## INTRODUCTION

Many microorganisms are capable of producing extracellular cellulases. Important among these are the filamentous fungi which digest cellulosic wastes rapidly through their high metabolic rates. While *Trichoderma* species and their mutants are regarded as the best producers of cellulases to-date (1,7) and much studies have been centred on them, other fungi like *Fusarium* and *Penicillium* species were also reported (8-11). In another direction, manipulation of culture conditions to maximize cellulase production has received much interest (12, 13, 14, 4, 5). Most of these works have focussed on the *Trichoderma* cellulase system, with comparatively fewer reports on other fungal species. Different methods are available for rapid screening of cellulolytic activities of fungi like insoluble cellulose incorporated into the agar medium and the clearing of the semi-opaque cellulose are used as an indication of cellulolytic activity (15). Mineral agar medium overlaid with cellulose-azure (0.05 g) as substrate was also used to screen extracellular cellulase activities and the blue dye is released from the cellulose azure and diffuses into the medium (16). Tan *et al.* (6) reported that forty strains of filamentous fungi including species of *Trichoderma*, *Aspergillus*, *Penicillium* and *Fusarium* secreted extracellular cellulases on cellulose-azure substrate. Twenty-nine fungal species isolated from dung samples of five herbivorous animals collected from arid and semi-arid areas of Iraq were capable of producing cleared zones on cellulose agar medium (17). More recently, Leinhos and Buchenauer (18) used the agar diffusion test to examine cellulolytic activity of *Verticillium chlamydosporium*, *V. gonioides*, *V. lecanii*, *V. psalliotae*, *V. tenuipes* and *Alternaria alternata*. In the present study cleared-zone technique, cellulose-azure method and agar diffusion test were used to screen extracellular cellulolytic activities of fungi isolated from Saudi Arabian soils (19).

## MATERIALS AND METHODS

### Chemicals

Carboxymethylcellulose-Na salt (CMC, low viscosity, Fluka, Switzerland), crystalline cellulose (Avicel) and cellulose-azure (Winlad, U.K.) were used for screening cellulolytic activities. All the other chemicals used were of Analar grade.

### Screening for Cellulolytic Ability of Fungi Organisms

Thirty isolates of the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Drechslera*, *Ulocladium*, *Chaetomium*, *Curvularia*, *Trichoderma*, *Cochliobolus*, *Rhizopus*, *Mucor*, *Phoma*, *Emericella*, and *Stachybotrys* were screened for extracellular cellulase activities. The fungal species were isolated from Saudi Arabian soil during the period of May-August 1993 (19). Modified Czapek Dox agar medium as described by Abdel-Hafez *et al.* (20) was selected to isolate fungi.

### Cleared-Zone Technique

The medium used for cleared-zone technique contained (g/L), 2 g  $\text{KH}_2\text{PO}_4$ ; 1.4 g  $(\text{NH}_4)_2\text{SO}_4$ ; 0.3 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ; 0.3 g  $\text{CaCl}_2$ ; 4 mg  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ; 1.6 mg  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ ; 1.4 mg  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ; 2 mg  $\text{CoCl}_2$ ; 0.8 g peptone; 2 ml Tween 80; 10 g cellulose; and 15 g agar. Urea (0.3 g) was added after autoclaving (21). Mycelial discs of 4 mm diameter cut from the edge of the active colonies were used as inocula. Quadruplicate sets of plates were inoculated for each fungus. The plates were incubated at 28°C for 7 days. At the

end of the incubation period the plates were flooded with a mixture of HCl/iodine solution which was prepared by mixing 1 ml 0.1 M HCl with 5 ml 1% (w/v)  $\text{I}_2$  in 2% (w/v) KI (15). A cleared yellow zone around the colony against a dark brown background was considered as a positive test for cellulase.

### Agar Diffusion Test

Fungi were grown in 250 ml flasks, each flask receiving 50 ml of the above cellulolytic medium (21) with 1% Avicel as a carbon source. Each flask was inoculated with three 4 mm mycelial discs from 7 day-old cultures and triplicate sets of cultures were prepared for each fungus. Cultures were incubated in an orbital incubator operating at 120 strokes/min at 28°C. After 7 days, the solid fractions (mycelia and residual cellulose) were separated from the culture broth by centrifuging the samples at 4,000 rpm for 10 min. The culture supernatant represented the enzyme source.

For the agar diffusion test, the following medium was used: 1% carboxymethyl cellulose and 2% agar in 0.1 mol/L phosphate buffer (pH 6.0). Enzyme preparations (0.2 ml) were added into agar holes (9 mm diam) and the plates incubated for 30 hr at 28±2°C. Triplicate sets of plates were prepared for each fungus. After incubation, the plates were overlaid with Cu-acetate solution 10% and the radius of the degradation zones was measured (18).

### Cellulose-Azure Method

The mineral agar medium plates contained per 100 ml,  $(\text{NH}_4)_2\text{SO}_4$  0.1 g;  $\text{KH}_2\text{PO}_4$  0.1 g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.02 g; Difco agar 1.5 g and overlaid with cellulose-azure (0.05 g) as a substrate (16). Fungal cellulolytic activities were assessed on the basis of their ability to utilize cellulose-azure and subsequently releasing the blue azure-dye into the basal mineral agar. The relative cellulolytic activity of each species was scored by comparing the intensity of the blue colour of the mineral medium with a standard blue colour scale of 1 to 10 (maximum) over an incubation period of 10 days. All tests were set up on the same day and periodically inspected for the release of the dye. Three replicates were set up for each isolate and uninoculated medium held under the same conditions served as control.

## RESULTS AND DISCUSSION

The objective of these experiments was to determine the cellulolytic ability in isolated fungi. Throughout this study different methods were used for comparative purposes and the tested isolates were screened for extracellular cellulase activities. All the isolated fungi had different abilities to produce extracellular cellulases.

From the cleared-zone technique it was difficult to determine the percent cleared-zone with respect to colony size as an indication of the enzyme activity level. The disadvantage of this method could be observed in the difficulty of observing the clearing zone. Most of the isolates did not show clearance in the visual scoring for strong cellulolytic activity (Table 1). In the cellulose-azure method, the results of the rapid screening for extracellular cellulase activity showed that overall tested fungal isolates secreted the enzyme. Some isolates caused the release of some of the blue dye from the cellulose within 3-4 days but others took considerably longer time. The final results were recorded after 10 days of incubation (Table 2).

**Table 1**  
Cellulolytic activity using cleared-zone technique

Fungal species	IMI Number	Cellulolytic Activity*
<i>Aspergillus fumigatus</i> Fres.	356162	++
<i>A. niger</i> van Tiegh.	356160	++
<i>A. flavus</i> Link	356149	++
<i>A. terreus</i> Thom		++
<i>Alternaria alternata</i> (Fr. :Fr.) Keissler		
<i>Alternaria</i> sp.		
<i>Curvularia</i> sp.		-
<i>Cochliobolus spicifer</i> R.R. Nelson		+
<i>C. lunatus</i> R.R. Nelson & F.A. Haasis	356138	+
<i>Chaetomium</i> sp.	356145	++
<i>Drechslera</i> sp.		++
<i>Emericella nidulans</i> (Eidam) Vuill.		++
<i>Fusarium moniliforme</i> Sheldon	356153	++
<i>F. solani</i> (Martius) Sacc.	356146	+
<i>Mucor</i> sp.	356159	++
<i>Penicillium chrysogenum</i> Thom.	356165	++
<i>P. griseofulvum</i> Dierckx	356166	++
<i>Penicillium</i> sp.		
<i>Phoma</i> sp.		+
<i>Rhizopus</i> sp.		-
<i>Stachybotrys</i> sp.		++
<i>Trichoderma viride</i> Pers. :Fr.		++
<i>T. harzianum</i> Rifai		++
<i>T. koningii</i> Oudem.	356151	++
<i>T. pseudokoningii</i> Rifai	356158	++
<i>Ulocladium atrum</i> Preuss	356164	+
<i>U. septosporum</i> (Preuss) E.G. Simmons	350894	+
<i>Ulocladium</i> sp.	356143	+
	356142	

IMI number = International Mycological Institute Number.  
 \*++ : strong cellulolytic activity; + weak cellulolytic activity;  
 -; no cellulolytic activity.

**Table 2**  
Intensity of blue azure dye released by fungal isolates on mineral agar medium

Fungal species	Days									
	1	2	3	4	5	6	7	8	9	10
<i>Aspergillus fumigatus</i> Fres.	0	1	4	6	7	8	8	9	10	10
<i>A. niger</i> van Tiegh.	0	0	1	1	2	3	3	4	5	5
<i>A. flavus</i> Link	0	0	1	2	3	4	5	5	6	7
<i>A. terreus</i> Thom	0	0	0	0	1	2	2	3	3	4
<i>Alternaria alternata</i> (Fr. :Fr.) Keissler	0	0	0	0	0	1	1	2	3	3
<i>Alternaria</i> sp.	0	0	0	0	0	1	2	3	4	4
<i>Curvularia</i> sp.	0	0	0	0	0	0	1	2	2	2
<i>Cochliobolus spicifer</i> R.R. Nelson	0	0	0	0	1	1	2	3	4	4
<i>C. lunatus</i> R.R. Nelson & F.A. Haasis	0	0	1	2	3	4	5	5	6	7
<i>Chaetomium</i> sp.	0	0	0	1	2	2	3	4	5	5
<i>Drechslera</i> sp.	0	0	0	1	2	3	3	4	4	4
<i>Emericella nidulans</i> (Eidam) Vuill.	0	0	0	0	1	2	2	3	4	4
<i>Fusarium moniliforme</i> Sheldon	0	0	0	1	2	3	4	4	5	6
<i>F. solani</i> (Martius) Sacc.	0	0	0	0	0	1	2	3	4	4
<i>Mucor</i> sp.	0	0	0	0	0	0	1	1	1	1
<i>Penicillium chrysogenum</i> Thom.	0	0	0	1	1	2	3	3	4	4
<i>P. griseofulvum</i> Dierckx	0	0	0	0	1	2	3	4	4	4
<i>Penicillium</i> sp.	0	0	0	0	1	1	2	3	4	4
<i>Phoma</i> sp.	0	0	0	0	0	0	1	1	2	3
<i>Rhizopus</i> sp.	0	0	0	0	0	0	1	2	1	1
<i>Stachybotrys</i> sp.	0	0	0	0	0	1	1	2	4	6
<i>Trichoderma viride</i> Pers. :Fr.	0	2	3	6	8	10	10	10	10	10
<i>T. harzianum</i> Rifai	0	0	0	4	5	6	7	7	8	8
<i>T. koningii</i> Oudem.	0	0	1	3	4	5	6	7	7	8
<i>T. pseudokoningii</i> Rifai	0	0	4	7	7	8	9	10	10	10
<i>Ulocladium atrum</i> Preuss	0	0	0	0	1	2	3	3	4	4
<i>U. septosporum</i> (Preuss) E.G. Simmons	0	0	0	1	2	3	4	5	6	6
<i>Ulocladium</i> sp.	0	0	0	0	0	1	2	2	3	4

Number 1-10 (maximum) intensity scale of the blue azure dye released from cellulose-azure into basal mineral azure medium through cellulolytic activity of fungal isolates.

Twenty isolates gave moderate results, seven showed moderate to strong activity, with three isolates of *Aspergillus fumigatus*, *Trichoderma viride* and *T. pseudo-*

*koningii* giving the most positive results. None, however, caused as fast a release of the dye as *T. viride* (Table 2). Some typical examples are shown in Fig. 1.



Fig. 1: Intensity of blue azure dye released from cellulose-azure into mineral agar medium through cellulolytic activity of *T. viride*.

All isolates were able to degrade carboxymethyl cellulose (CMC) in agar diffusion tests by production of cellulolytic exoenzyme (Table 3). *Trichoderma viride*, *T. pseudokoningii*, *Aspergillus fumigatus* and *A. flavus* produced large lytic zones (7-8 mm in radius) around the fungal colony on CMC agar, and the maximum (10 mm) was seen in *T. viride*. The other isolates produced smaller lytic zones (2-5 mm) beyond the fungal colonies. Heat-treated enzyme preparations used as controls proved to be inactive in the agar diffusion test.

**Table 3**  
Cellulolytic activity of 7-day-old culture filtrates of fungal isolates  
(Agar diffusion test).

Fungal species	IMI Number	Cellulolytic Activity*
<i>Aspergillus fumigatus</i> Fres.	356162	8.0 ± 0.3
<i>A. niger</i> van Tiegh.	356160	4.0 ± 0.2
<i>A. flavus</i> Link	356149	7.0 ± 0.1
<i>A. terreus</i> Thom	-	3.0 ± 0.4
<i>Alternaria alternata</i> (Fr. :Fr.) Keissler	-	2.0 ± 0.3
<i>Alternaria</i> sp.	-	2.0 ± 0.3
<i>Curvularia</i> sp.	-	2.0 ± 0.4
<i>Cochliobolus spicifer</i> R.R. Nelson	356138	4.0 ± 0.8
<i>C. lunatus</i> R.R. Nelson & F.A. Haasis	356145	5.0 ± 0.7
<i>Chaetomium</i> sp.	-	4.0 ± 0.2
<i>Drechslera</i> sp.	-	3.0 ± 0.1
<i>Emericella nidulans</i> (Eidam) Vuill.	356153	4.0 ± 0.5
<i>Fusarium moniliforme</i> Sheldon	356146	5.0 ± 0.1
<i>F. solani</i> (Martius) Sacc.	356159	4.0 ± 0.1
<i>Mucor</i> sp.	-	0.0
<i>Penicillium chrysogenum</i> Thom.	356165	4.0 ± 0.3
<i>P. griseofulvum</i> Dierckx	356166	4.0 ± 0.11
<i>Penicillium</i> sp.	-	2.0 ± 0.3
<i>Phoma</i> sp.	-	0.0
<i>Rhizopus</i> sp.	-	0.0
<i>Stachybotrys</i> sp.	-	4.0 ± 0.7
<i>Trichoderma viride</i> Pers. :Fr.	356151	10.0 ± 1.1
<i>T. harzianum</i> Rifai	356158	5.0 ± 0.3
<i>T. koningii</i> Oudem.	356164	5.0 ± 0.1
<i>T. pseudokoningii</i> Rifai	350894	8.0 ± 0.7
<i>Ulocladium atrum</i> Preuss	356143	4.0 ± 0.3
<i>U. septosporum</i> (Preuss) E.G. Simmons	356142	5.0 ± 1.0
<i>Ulocladium</i> sp.	-	2.0 ± 0.6

\*Radius of the lysis halos (mm) with carboxymethylcellulose as enzyme substrate in the agar diffusion assay (n=3); (mean ± SD); IMI number = International Mycological Institute Number.

In the agar diffusion test, only *T. viride*, *T. pseudokoningii* and *A. fumigatus* exhibited high cellulolytic activity. Also,

in cellulose-azure method, *Trichoderma* species were generally the earliest cellulase producers (Table 2) showing observable dye release between 3-4 days after inoculation. Similar observations have also been made (6). The levels of enzyme activity were also high, and the blue intensity at the end of 10 days ranging from 7 to the maximum 10 was seen in *T. viride* and *T. pseudokoningii*, but in *T. koningii* and *T. harzianum* were less than 10. Dye release was slow between 4-5 days after inoculation with *Aspergillus* spp. comparable with *Trichoderma* spp., and only one species of *Aspergillus* (*A. fumigatus*) exhibited maximum cellulase activity of 10 at the end of 10 days of incubation. Although species of *Penicillium* have been widely isolated from nature by the use of cellulose media (22-24), few have been assessed for their cellulolytic ability. Highly cellulolytic species reported including *P. verruculosum* (11) and *P. funiculosum* were a source of commercial production of cellulase (10). However, throughout this study, *Penicillium* spp. were considered as moderately cellulolytic. *Aspergillus flavus*, *F. moniliforme*, *U. septosporum*, *C. lunatus*, *Chaetomium* sp., *T. harzianum* and *T. koningii* show moderate to strong activities of dye release which were detected slightly between 4-5 days. Other species have also been demonstrated to degrade cellulose-azure, but at a less extent comparable with *Trichoderma viride*, *T. pseudokoningii* and *A. fumigatus*.

## CONCLUSIONS

Cellulolytic enzymes were examined in the agar diffusion test. Although a survey of the extracellular enzyme production by filamentous fungi using the qualitative techniques serves as a convenient way of demonstrating some types of extracellular enzymes in a relatively short time, most enzymes can be detected visually only beyond the periphery of the colony. Enzymes may be produced directly under the fungal colony and their detection is especially difficult. Some others may be bound to the fungal cell wall. Zone clearing in the cellulose Czapek agar plate was indistinct and extremely difficult to identify. So comparison of data suggests that some caution should be made to the use of cellulose agar test medium, otherwise an inaccurate view of enzyme production by certain fungi may result. However, a preliminary study with the isolates of a fungal species revealed that the production of cellulolytic enzymes was induced by cellulose. The isolates may vary in the production of enzymes, while the isolates of *T. viride*, *T. pseudokoningii* and *A. fumigatus* exhibited consistent high cellulolytic activities.

## REFERENCES

- [1] Mandels, M., Weber, J. and Parizek, R., 1971. Enhanced cellulase production by a mutant of *Trichoderma viride*. *Applied Microbiology*, 21: 152-154.
- [2] Herr, D., Luck, G. and Dellweg, H., 1978. Formation of cellulases and degradation of cellulose by several fungi. *Journal of Fermentation Technology*, 56: 273-278.
- [3] Halliwell, G., 1965. Hydrolysis of fibrous cotton and precipitated cellulose by cellulolytic enzymes from soil micro-organisms. *Biochemical Journal*, 95: 270-281.
- [4] Rubidge, T., 1984. Extracellular cellulase activity for *Trichoderma viride* grown in the presence of glucose. *Transactions of British Mycological Society*, 85: 522-524.
- [5] Sandhu, D. and Kalra, M.K., 1985. Effect of cultural

- conditions on production of cellulases in *Trichoderma longibrachiatum*. Transactions of British Mycological Society, 84: 251-258.
- [6] **Tan, T.K., Yeoh, M.H., Tan, M.L. and Koh, S.K., 1987.** Cellulolytic activities of some filamentous fungi. Journal of the Singapore National Academy of Science, 16: 11-16.
- [7] **Wood, T.M. and Philips, D.R., 1969.** Another source of cellulase. Nature (London), 222-986.
- [8] **Chang, W.S. and Usami, S., 1969.** Production and utilisation of *Trichoderma cellulase* by submerged culture. Journal of Fermentation Technology, 47: 447-455.
- [9] **Boretti, G., Garofano, L., Montencuccii, P. and Spalia, G., 1972.** Cellulase production with *Penicillium iriensis*. Archive Microbiology, 92: 189-200.
- [10] **Parr, S.R., 1983.** Some kinetic properties of the  $\beta$ -glucosidase (cellobiase) in a commercial cellulose product from *Penicillium funiculosum* and its relevance in the hydrolysis of cellulose. Enzyme and Microbial Technology, 5: 457-462.
- [11] **Szakacs, G., Reczey, K., Hernadi, P. and Dobozi, M., 1981.** *Penicillium verruculosum* WA 30, a new source of cellulase. European Journal of Applied Microbiology and Biotechnology, 11: 120-124.
- [12] **Stewart, J.C. and Parry, J.B., 1981.** Factors influencing the production of cellulase by *Aspergillus fumigatus*. Journal of General Microbiology, 125: 33-39.
- [13] **D'Souze, J. and Volfova, O., 1982.** The effect of pH on the production of cellulase by *Aspergillus fumigatus*. European Journal of Applied Microbiology and Biotechnology, 16: 123-125.
- [14] **Ferchak, J.D. and Pye, E.K., 1983.** Effect of cellobiose, glucose, ethanol and metal ions on the cellulase enzyme complex of *Thermonospora fusca*. Biotechnology and Bioengineering, 25: 2855-2864.
- [15] **Yeoh, H.H., Khew, E. and Lim, G., 1985.** A simple method for screening of cellulolytic fungi. Mycologia, 77: 161-162.
- [16] **Smith, J.E., 1977.** Rapid tube test for detecting fungal cellulase production. Applied and Environmental Microbiology, 33: 980-981.
- [17] **Taj-Aldeen, S.J., Al-Habbeeb, E.K. and Abdulla, S.K., 1990.** Cellulolytic activity of coprophilous fungi. Cryptogamic Botany, 2: 25-29.
- [18] **Leinhos, G.M.E. and Buchenauer, H., 1992.** Hyperparasitism of selected fungi on rust fungi of cereal. Journal of Plant Diseases and Protection, 99: 482-498.
- [19] **Khiyami, M.A., 1994.** Physiological studies on the cellulolytic enzymes of some filamentous fungi isolated from various soils of Saudi Arabia. M.Sc. Thesis. Botany and Microbiology Department; King Saud University, Riyadh, Saudi Arabia.
- [20] **Abdel-Hafez, A.I.I., Mazen, M.B. and Galal, A.A., 1990.** Glycophilic and cellulose-decomposing fungi from soils of Sinai Peninsula, Egypt, Arab Gulf Journal of Scientific Research, 8: 153-168.
- [21] **Mandels, M., Sternberg, D. and Andreotti, R., 1975.** Symposium on enzymatic hydrolysis of cellulose. Aulanko, Finland. Baily, M., Enari, T.M. and Linko, E. (eds). pp. 81-109. Denver Bookbinding Co., Denver.
- [22] **Abdel-Hafez, S.I.I., and Abdel-Kader, M.I.A., 1980.** Cellulose-decomposing fungi of barley grains in Egypt. Mycopathologia, 70: 77-82.
- [23] **El-Kady, I.A., Abdel-Hafez, S.I.I. and Moubasher, M.H., 1981.** Survey on the cellulose-decomposing fungi of wheat straw in Egypt. Mycopathologia, 76: 59-64.
- [24] **Moubasher, A.H., Abdel-Hafez, S.I.I. and El-Maghraby, O.M.O., 1985.** Studies on soil mycoflora of wadi Bir-El-Ain, Eastern Desert Egypt. Cryptogamic Mycology, 6: 129-143.