Bioconversion of Straw into Improved Fodder: Fungal Flora Decomposing Rice Straw

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The fungal flora decomposing rice straw were investigated all over the soil of Sharkia Province, east of Nile Delta, Egypt, using the nylon net bag technique. Sixty-four straw-decomposing species belonging to 30 genera were isolated by the dilution plate method in ground rice straw-Czapek's agar medium at pH 6. The plates were incubated separately at 5°C, 25°C and 45°C, respectively. Twenty nine species belonging to 14 genera were isolated at 5°C. The most frequent genus was *Penicillium* (seven species), and the next frequent genera were *Acremonium* (three species), *Fusarium* (three species), *Alternaria*, *Chaetomium*, *Cladosporium*, *Mucor*, *Stachybotrys* (two species) and *Rhizopus stolonifer*. At 25°C, 47 species belonging to 24 genera were isolated. The most frequent genus was *Aspergillus* (nine species), and the next frequent genera were ranked by *Penicillium* (five species), *Chaetomium* (three species), *Fusarium* (three species). Each of *Alternaria*, *Cladosporium*, *Mucor*, *Myrothecium* and *Trichoderma* was represented by two species. At 45°C, 15 species belonging to seven genera were isolated. These were seven species of *Aspergillus*, two species of *Chaetomium* and two species of *Emericella*, while *Humicola*, *Malbranchea*, *Rhizomucor* and *Talaromyces* were represented by one species respectively. The total counts of fungi the genera, and species per gram of dry straw were significantly affected by incubation temperature and soil analysis (P < 0.05).

KEYWORDS: Bioconversion, Fodder, Fungal decomposition, Rice straw

Rice (Oryza sativa L), one of the world's leading crops, is cultivated in about 2×10^6 feddans in Egypt and the production of rice straw reaches about 8×10^6 tons per year. Straw is usually either burnt in the field causing environmental hazards such as respiratory diseases or disposed in a way that does not benefit the farmers to its maximum extent (Arai et al., 1998; Samar et al., 1999; Torigoe et al., 2000). Fungi are the most important group among microbial agents for straw decomposition (Hudson, 1972; Srinivason, 1979; Harper and Lynch, 1982a; Yananobe et al., 1994; Morais et al., 1999; Tengerdy and Szakacs, 2003). There have been many surveys of cellulose-decomposing fungi but most fungi were isolated directly from soil or other sources on pure cellulose (Abdel-Hafez et al., 1978; Abdel-Hafez and Abdel-Kader, 1980; Mazen et al., 1980; Abdel-Hafez, 1982; Abdel-Kader et al., 1983; Moubasher et al., 1985). Surveys on wheat straw and other cultural wastes were conducted by other workers (El-Nawawy, 1972; El-Kady et al., 1981; Abdel-Hafez et al., 1990). Moubasher et al. (1985) isolated 19 species (one variety) belonging to 13 genera from wheat and broad bean straw compost at 45°C. However, few studies were focused on rice straw. Coronel et al. (1991) found that Aspergillus fumigatus was the most active cellulase producer on rice straw substrate among 144 tested strains of thermophilic lignocellulose-degrading fungi. Rai et al. (2001) isolated 42 mesophilic species from litter of rice

straw.

Research on the bioconversion of these agricultural residues into a microbial biomass as an improved feed supplement has been conducted in this study. The present investigation aimed the mycoflora inhabiting rice straw at 5°. 25° and 45°C in different localities.

Materials and Methods

Samples and soil analysis. Nylon net bag technique (House and Stinner, 1987; Wise and Schaefer, 1994) was used in this study. Thirty nylon bags each containing 10g of rice straw chopped to lengths of 5~6 cm, were prepared. These bags were distributed in different places throughout 10 areas (three bags in each area) of Sharkia Province of Egypt, in which large quantities of rice straw are produced. These sampling areas were; Abo-Kabir, Diarb-Negm, Hehia, Mashtool, Menia El-Kamh and Zagazig soils where were cultivated with Triticum vulgare and Trifolium alexandrinum after harvesting of paddy rice, Orvza sativa. Abo-Hamad and Fakoos areas were Mangifera indica orchards while Belbeis and Kafr-Sakr were orchards of Citrus sinensis. The bags were buried 5~10 cm from the soil surface for 10 days, then collected again for investigation.

Soil samples collected from the same localities at which nylon net bags were buried, were analyzed chemically for total soluble salts and organic matter content (Jackson, 1962). The soil texture was determined by the

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sieve method (Piper, 1947) using a standard, Rot-Top electric sieve shaker, VEB Metall Weberei Neustadi, Orta D.D.R. The reaction of the soil was measured using the glass electrode pH-mater (Richards, 1954).

Estimation of cellulose decomposing fungi. Fungi inhabiting rice straw were estimated using dilution plate method as described by Johnson et al. (1959). One ml of the straw suspension was introduced to each agar plate using Menzies dipper (1957) as recommended by Watson (1960) and Moubasher (1963). Modified Czapek's agar medium was used in which ground rice straw replaced sucrose (20 g/l) and to which rose Bengal (1/15000) was added as a bacteriostatic agent (Smith and Dawson, 1944), and the pH of the medium was adjusted to 6.0. Fifteen plates for each bag were used and incubated at 5°. 25° and 45°C (five plates at each temperature degree) for 15 days. The plates were examined daily. The average number of colonies per dish was multiplied by the dilution factor to obtain the number of colonies/g dry straw in the original rice straw sample.

Fungal identification. The fungal colonies appeared were picked up on Czapek's 0.5% yeast extract, malt extract and/or potato dextrose agar media, purified using spore technique and identified in this laboratory by consulting the following works: Gams *et al.*, 1998; Kubicek and Harman, 1998; Moubasher, 1993; Kitch and Pitt, 1992; Pitt, 1986; Domsch *et al.*, 1980; Pitt, 1979; Booth, 1977; Raper and Fennell, 1977; Ellis, 1971; Rifai, 1969; Raper and Thom, 1968; Cooney and Emerson, 1964.

Statistical analysis. The obtained data were conducted to one-way ANOVA and multiple-way ANOVA by Snedecor and Cochran (1982) and differences between means were done at the 5% probability level using Duncan's new multiple range tests (Duncan, 1955). Bivariate corre-

lation matrix of the obtained data was done using by SPSS software program (ver. 8) as described by Dytham (1999).

Results and Discussion

The straw dilutions (of each nylon net bag) used for estimation of rice straw-degrading fungi were 10⁻⁴ in the case of incubating the plates at 5° and 45°C, and 10⁻⁵ for 25°C. Rai *et al.* (2001) used dilution 10⁻⁴ for isolation of litter decomposing mycoflora of rice straw using nylon net bag technique. They also found that dilution plate method yielded maximum number of fungi in comparison with direct observation and damp chamber incubation methods.

Results in Tables 1 and 2 show that the total count of fungi per gram of dry straw was significantly affected (P < 0.05) by incubation temperature, pH value (-ve r), organic matter (+ve r) and total soluble salts (-ve r) of the soil where the nylon net bag was buried. The fungal-rich soils were sample no. 10, 9, 8, 4, 6 and 2, which are characterized by clay type, to be slightly acidic to neutral (6.9~7.2 pH-value) with high organic matter content (1.87~ 1.53%), and low total soluble salts (0.46~0.63%), and cultivated with Triticum and Trifolium. However the fungalpoor samples were soil sample no. 3 and 5 which are sandy, alkaline (7.8 and 7.7 pH), with low organic matter content (0.25 and 0.41%), high in total soluble salts (2.20 and 1.67%) and cultivated with Citrus and Mangifera. Abdel-Hafez (1978) and Helal (1993) found that the total population of fungi was influenced by the content of the total soluble salts of the soil. The highest fungal population was recorded from sample no. 10 (Zagazig area) which showed, 7.2×10^4 , 7.1×10^5 and 5.8×10^4 colony/g dry straw, while the lowest fungal population with 1.4 × 10^4 , 7.8×10^4 and 1.7×10^4 colony/g dry straw from sample number 3 (Belbeis area) at incubation temperature 5°. 25° and 45°C, respectively. Abdel-Sater and El-Said (2001)

Table 1. Characterization of soil samples tested and rice straw-decomposing fungi

Sample	Locality	Soil analysis			Total cou	No. of genera			No. of species					
no.	Locality -	Soil type	pH-value	OM%	TSS%	5°C	25°C	45°C	5°C	25°C	45°C	5°C	25°C	45°C
1	Abo-Hamad	Clay-sandy	7.5 b	0.85 c	0.91d	31297 ^x _d	236184 ^y	36184 ^x _{bc}	10° bc	15 ^y _e	4 ^z _b	21 ^x _{de}	30° _e	10 ² _b
2	Abo-Kabir	Clay	7.2 c	1.53 b	0.63 e	52472° to	453752 ^y e	44793° ab	9^{x}_{cd}	17^{y}_{d}	4 ² _b	20^{x}_{c}	33^{y}_{cd}	10^{z}_{b}
3	Belbeis	Sandy	7.8 a	0.25 e	2.20 a	13613°	77952 ^y	16987° _d	7°.	8^{y}_{h}	4 ^z _b	12° _g	12 ^x	7°.
4	Diarb-Negm	Clay	6.9 cd	1.77 ab	0.53 ef	53520° bc	603839 ^y c	54450° a	11^{x}_{ab}	21 ^y _b	6^{z}_{a}	24 ^x _{ab}	39 y	12 z
5	Fakoos	Sandy	7.7 ab	0.41 de	1.76 c	25713° de	221059 ^y s	29668 ^x cd	9^{x}	12^{y}_{g}	4^{z}_{b}	15° _f	$22^{\frac{y}{g}}$	8° c
6	Hehia	Clay	7.2 c	1.77 ab	0.60 ef	46422° c	524724 ^y d	43979° ab	10 s	18 ^y c	5° ab	20^{x}_{e}	$32^{\frac{y}{d}}$	8° c
7	Kafr-Sakr	Clay-sandy	7.6 ab	0.62 cd	2.06 b	16754 ^x _{de}	186155 ^y	15241 ^x _d	8_x^{q}	13^{y}_{f}	4 ^z _b	14^{x}_{f}	25 ^y f	7° _c
8	Mashtool	Clay	7.1 c	1.77 ab	0.47 f	59104 ^x	596859	54101° _a	11 ^x ab	18 ^y c	5^{z}_{ab}	23° bc	34 °.	$12^{\frac{z}{a}}$
9	Menia El-Kamh	Clay	7.0 cd	1.80 a	0.46 f	66434 ^x _{ab}	648051 ^y	54567° _a	12° _a	21^{y}_{b}	6° a	22^{x}_{cd}	41 ^y a	12° a
10	Zagazig	Clay	6.8 d	1.87 a	0.46 f	72019 ^x	705051 ^y	58290° a	11 ^x ab	23 ^y a	6^{z}_{a}	25° a	42 y	12^{z}_{a}
	Mean					43677	425363	40838	10	17	5	20	31	10

¹⁻The same letter in the same column/ row means not significant at p < 0.05.

²⁻a, b, c, ... for column comparison and x, y, z for row comparison.

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Table 2. Pearson correlation matrix (r) and probability showing the relationship between soil analyses (pH, OM and TSS) and no. of genera, no. of species and total count at different incubation temperatures

6.11		Total count			No. of gener	a	No. of species					
Soil analysis	Incubati	on tempera	ture °C	Incubati	on tempera	ture °C	Incubation temperature °C					
anarysis	5	25	45	5	25	45	. 5	25	45			
OM	0.926**	0.956**	0.914**	0.783**	0.918**	0.607**	0.872**	0.898**	0.757**			
PH	0.882**	0.903**	0.864**	0.724**	0.903**	0.596*	0.885**	0.885*	0.746**			
TSS	0.842**	0.842**	0.842**	0.829**	0.893**	0.560*	0.899**	0.899**	0.812**			

^{*}Correlation is significant at the 0.01 level.

identified twenty three species belonging to 11 genera at 28°C from 30 samples of rice straw from Qena Governorate (South Valley), Egypt with total count 7.3×10^3 colonies/mg.

Sixty-four species belonging to thirteen genera were

collected in the present investigation (Table 3). The most diverse fungal flora was obtained at 25° C (24 genera and 47 species) with total fungal count 4.3×10^{5} colony/g dry straw, followed by 14 genera and 29 species at 5° C with 3.6×10^{4} colony/g dry straw, while only 7 genera and 15

Table 3. Characterization of rice straw-decomposing fungi isolated at 5°, 25° and 45°C in rice straw- Czapek's agar medium

	Incubation temperature °C 5 25 45												
Genus		5					25			TNS			
	NS	NCI	OR	TC	NS	NCI	OR	TC	NS	NCI	OR	TC	-
Acremonium	3	23	H	2815	1	3	R	1280	_		-		3
Alternaria	2	30	H	5433	2	30	Н	20710	-	_	_	-	2
Aspergillus	_		_	_	9	30	Н	141827	7	30	Н	24584	10
Botrytis	1 .	1	R	23	_	_	-	_	-	_	_	- ,	1
Botrytrichum	- "	_	. –	_	1	15	M	11984	-	_	_	_	1
Chaetomium	2	27	Н	3048	3	21	Н	16638	2	21	Н	1967	4
Cladosporium	2	30	Н	6341	2	25	Н	30134		_	-		2
Cochliobolus	_	_	_	_	2	11	M	6399	-	_	_		2
Colletotrichum	_	_		_	1	2	R	349	-	_	_	· -	1
Emericella	_	_	_	_	2	13	M	6400	2	16	H	1257	2
Epicoccum	1	7	L	81	1	3	R	698	_	_			1
Fusarium	3	29	Н	2827	3	26	Н	33973	_	_	_	-	4
Gliocladium	_	_	_	_	1	12	M	2094		_	_	_	1
Humicola	_	_	_	_	1	9	M	3723	1	13	M	593	1
Malbranchea	_	_	_	_	_	_	-	_	1	3	R	70	1
Mucor	2	28	Н	2641	2	26	Н	28854		_	_	_	2
Mvrothecium	_	_	_	_	2	22	Н	24782	_	_	_	_	2
Nigrospora	1	5	L	81	1	3	R	698	_	_	_	_	1
Oidiodendron	1	1	R	12		-	_	_	_	_	_	_	1
Penicillium	7	30	H	5573	- 5	25	H	35369	_	_	_	_	9
Phoma	_	_	_	_	Ī	11	M	3839	_	_	_	-	- 1
Pestalotia	1	1	R	23	_	_		_	-	_		_	1
Rhizomucor	_	_	_	_	_	_		_	1	30	Н	11914	1
Rhizopus	1	28	Н	1827	1	11	M	7330	_	_	_	_	1
Scopulariopsis	_	_	_	_	ì	3	R	582		_	_ '	_	1
Stachybotrys	2	30	Н	5376	1	13	M	5468	_	_	_	_	2
Talaromyces	_		_	-	_	_	-	-	1	10	M	314	1
Trichoderma	_	_	-		2	26	Н	36300	_		_	_	2
Trichothecium	_	_	_	_	1	9	M	5236	_		_	_	1
Verticillium	_	-	_	_	1	2 -	R	233	-	, —,		-	1
No. of genera No. of species		14 29		36101		24 47		424900		7 15		40699	.64

NS: number of species, NCI: number of cases of isolation, OR: occurrence remarks, TC: total count, TNS: total number of species.

^{**}Correlation is significant at the 0.001 level.

H=high occurrence isolated more than is 15 cases (out of 30).

M = moderate occurrence from 8 to 15 cases.

L = low occurrence from 4 to 7 cases.

R = rare occurrence, less than 4 cases.

species isolated at 45°C with the total fungal count 4.1 × 10⁴ colony/g dry straw. Most of the genera which appeared at 5°C were also occurred on 25°C except Botrytis, Oidiodendron and Pestalotia. These genera can grow at 25°C (Helal, 2005) but their slow growth may be prevented in the presence of rapidly growing mesophilic species. Results in Tables 3 and 4 also indicated that at 45°C the following genera appeared: Chaetomium thermophilum, Humicola grisea var thermoidea (Scytalidium thermophilum), Malbranchea sulfurea (M. cinnamomea), Rhizomucor pusillus and Talaromyces thermophilus. They are thermophilic, while seven species of Aspergillus, the two isolates of Emericella nidulans (Aspergillus nidulellus) and Chaetomium spirale appeared at both 25° and 45°C i.e. they are thermotolerant. These two groups were previously described by Domsch et al. (1980), Moubasher (1993) and Mouchacca (1997 and 2000a and b).

As shown in Table 4 the most frequent genus which appeared during this study was; Aspergillus (10 species), and the next frequent fungal genera were ranked by Penicillium (9 species), Chaetomium and Fusarium (4 species), Acremonium (3 species), Alternaria, Cladosporium, Cochliobolus, Emericella, Humicola, Mucor, Myrothecium, Stachybotrys and Trichoderma (2 species). Members of Aspergillus, Penicillium and Trichoderma were also found to be the most prevalent fungi isolated from rice straw as xylan decomposing fungi (Abdel-Sater and El-Said, 2001).

Aspergillus represented 100% of the samples at 25° and 45°C, constituting 33.4% and 60.4% of total fungi, respectively, but did not appear at 5°C. A. awamori, A. flauvs, A. fumigatus, A. niger and A. terreus were of high occurrence at both 25°C and 45°C, while A. flavus var columinaris was of low occurrence at both temperatures. At

25°C A. ochraceus and A. sydowii were of moderate occurrence, while A. tamarii was of rare occurrence. At 45°C A. ustus was of rare occurrence and represented 10% of the samples, and can grow at 25°C (Helal, 2005). The six species isolated at 45°C were thermotolerant fungi as mentioned above. These ten species of Aspergillus were isolated previously on cellulose medium from different sources (El-Kady et al., 1981; Abdel-Kader, 1983; Abdel-Halez et al., 1990; Rai et al., 2001).

Penicillium was the second most common genus isolated at 5°C and 25°C, but not at 45°C, and represented 100% and 83.3% of the samples constituting 15.4 and 8.3% of the total count of fungi, respectively. P. citrinum, P. oxalicum were of high occurrence and P. corylophilum was of low and moderate occurrence at both 5°C and 25°C, respectively. On the other hand, P. canescens was of low occurrence, P. chrysogenum was of high occurrence, P. hrequei was of rare occurrence, and P. verrucosum was of moderate occurrence at 5°C, while P. janthinellum and P. rubrum appeared only at 25°C with rare occurrence. Penicillium species were also isolated frequently from agricultural residue, compost and manures in Egypt (El-Dohlob et al., 1985) and other parts of the world (Satyanarayana et al., 1988; Banerjee et al., 1995).

Chaetomium came third after Aspergillus and Penicillium. It was isolated at 5°, 25° and 45°C and represented 90, 73 and 73% of the samples constituting 8.4, 3.9 and 4.8% of total count of fungi, respectively. C. cochliodes was of moderate occurrence and C. globosum was of high occurrence at 5° and 25°C, C. spirale was of moderate occurrence at 25° and 45°, while C. thermophilum, a thermophilic fungus, occurred only at 45°C. Soytong (1991) found that some Chaetomium species isolated from the

Table 4. Fungi recovered in ground rice straw-Czapek's agar at 5°, 25° and 45°C in 30 rice straw samples using Nylon net bag technique and dilution plate method

	Incubation temperature °C										
Species		5			25			45			
	TC	NCI	OR	TC	NCI	OR	TC	NCI	OR		
Acremonium fusidioides (Nicot) Gams	919	13	M		_			_	_		
A. kiliense Grütz	1815	23	Н	_	-	_	-	-	_		
A. strictum W.Gams	81	5	L	1280	3	R		-	_		
Alternaria alternata (Fr.) Keiss.	4549	30	Н	15940	22	H	-	-	-		
A. tenuissima (Kunze ex Pers.) Wilts.	884	17	Н	4770	12	M		-	_		
Aspergillus awamori Nakaz.	_	_	-	12915	22	H	989	24	Н		
A. flavus Link var flavus		_	-	24666	27	H	2955	30	Н		
A. flavus var. columnaris Raper & Fennell	_	_	-	3723	7	L	58	3	R		
A. fumigatus Fr. var. fumigatus	_	_	_	25596	26	H	3560	25	Η		
A. niger Tiegh. var. niger	_	_	_	26760	27	H	7784	30	Н		
A. ochraceus Wilh.	_	_	-	11402	12	M	-	-	_		
A. sydowii (Bain & Sart.) Thom & Church	_	_	_	6748	12	M	-	_			
A. tamarii Kita	_	_	_	814	2	R		_	_		
A. terreus Thom var. terreus	_	_	_	29203	25	Н	9191	30	Н		
A. ustus (Bain) Thom & Church	_	_	-	_	_	-	47	3	R		

Table 4. Continued

	Incubation temperature °C										
Species	5			25			45				
	TC	NCI	OR	TC	NCI	OR	TC	NCI	OR		
Botrytis cinerea Pers.	23	1	R		_	-	_	_			
Botrytrichum piluliferum Sacc. & Marchal	_	_	_	11984	15	M	-	_			
Chaetomium cochliodes Pall.	768	15	M	1862	8	M	_	_	_		
C. globosum Kunze ex Stend.	2280	27	Н	8959	18	H	_	_			
C. spirale Zopf	_		_	5817	14	M	1164	13	M		
C. thermophilum La Touche	_		_	-	_		803	9	M		
Cladosporium cladosporioides (Fresen.) de Vries	6341	30	Н	10239	14	M		-	_		
C. herbarum (Pers.) Link	7574	30	Н	19895	23	Н	_	_	_		
Cochliobolus lunatus Nelson & Haasis		_	_	4421	10	M	_	_	_		
C. sativus (Ito & Kurib.) Drechsler ex Dastur		_		1978	5	L	_	_	_		
Colletotrichum dematium (Pers. ex Fr.) Grove		_	_	349	2	R	-',	_			
Emericella nidulans (Eidam) Vuillemin var. Lata Subramanian		_		5236	12	M	1001	14	M		
E. quadrilineata (Thom & Raper) Benj.	_		_	1164	5	L	256	7	L		
Epicoccum nigrum Link	81	7	L	698	3	R	_	_	_		
Fusarium moniliforme Sheld.	256	12	M	2792	7	M	_				
F. oxysporum Schlecht	230	-		19197	24	Н		'	_		
F. pallidoroseum (Cooke) Sacc.	442	17	Н	-	2 -	_					
F. solani (Mart.) Sacc.		28	H	11984	17	– H	_	_			
Gliocladium catenulatum Gilm. & Abbott	2129							_			
	_		_	2094	12	M	_				
Humicola grisea Traaen var. grisea	_	_	_	3723	9	M	-	12			
H. grisea var. thermoidea Cooney & Emers.	_		_	-	_		593	13	M.		
Malbranchea sulfurea (Miehe) Sigler& Carmich.		-	-	2.400	-	-	70	3	R		
Mucor circinelloides Van Tiegh.	419	13	M	3490	11	M	_		_		
M. racemosus Fresen.	2222	28	Н	25364	25	Н		-	_		
Myrothecium roridum Tode		_	-	22920	22	Н	-	-	. –		
M. verrucaria (Alb. & Schwein.) Ditmar	-	_	-	1862	5	L		-	-		
Nigrospora sphaerica (Sacc.) Mason	81	5	L	698	3	R	-	_			
Oidiodendron griseum Robak	12	1	R	_	_	-	_	-	. –		
Penicillium canescens Sopp	128	7	L	_	_	-	_		-		
P. chrysogenum Thom	1757	27	Н	_	-	-	-		_		
P. citrinum Thom	791	19	Н	15241	21	Η	-	-			
P. corylophilum Dierckx	163	7	L	4072	9	M	_	_	_		
P. herquei Bain. & Sart.	23	2	R	_	***	_	_	-	_		
P. janthinellum Biourge	_	_		233	1	R	_		-		
P. oxalicum Currie & Thom	2315	29	Н	15125	20	Η		_			
P. rubrum Stoll	_	-	_	698	3	R	-	-	-		
P. verrucosum Dierckx var. verrucosum	396	14	M	_	_		_	_	_		
Phoma herburum Peyronel	_	_	_	3839	11	M		_	_		
Pestalotia sp.	23	1	R	_	_	_	_	_	_		
Rhizomucor pusillus (Lindt) Schipper	****	_	_		_		11914	30	Н		
Rhizopus stolonifer (Lindt) Schipper	1827	28	Н	7330	11	M	_	_	_		
Scopulariopsis brevicaulis (Sacc.) Bain.	_	_	_	582	3	R		_	_		
Stachybotrys chartarum (Ehrenb. ex Link) Hugh.	3165	29	Н	5468	13	M	_	_			
S. elegans (Pidopl.) Gams.	2211	30	H	-	_	_		_	_		
Talaromyces thermophilus Stolk		_	_		_	_	314	10	M		
Trichoderma harzianum Rifai	-	_		13031	19	Н	_	_			
T. koningii Oudem.	_		_	23269	26	Н		_			
Trichothecium roseum (Pers.) Link ex Gray	_	_	_	5236	9	M		_			
Verticillium catenulatum (Kamyschko ex Barron & Onions) Gams	_	_	_	233	2	R	_	_	_		
Sterile mycelia	_	_	_	465	3	R	_				

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TC: total count, NCI: number of cases of isolation, OR: occurrence remark.

H = high occurrence isolated more than is 15 cases (out of 30).

M = moderate occurrence from 8 to 15 cases.

L = low occurrence from 4 to 7 cases.

R = rare occurrence, less than 4 cases.

rhizosphere of some economical plants can degrade rice husks, rice straw and paper.

Fusarium occupied the fourth place was isolated at 5° and 25°C but did not appear at 45°C and represented 96.7 and 86.7% at both temperatures constituting 7.8 and 8.0% of the total count of fungi, respectively. F. moniliforme was of moderate occurrence and F. solani was of high occurrence at both 5° and 25°C. F. oxysporum was of high occurrence at 25°C only, while F. pallidoroseum (F. semitectum) was of high occurrence at 5°C. El-Kady et al., (1981) isolated F. oxysporum and F. moniliforme in high occurrence on celluose medium from wheat straw. Also, Rai et al. (2001) reported that F. semitectum was dominant on rice straw.

Acremonium consisted of three species. A. fusidioides was of moderate occurrence and A. kiliense was of high occurrence only at 5°C. A. strictum appeared at 5 and 25°C in low and rare occurrence, respectively. Acremonium represented 76.7 and 10% of the samples constituting 7.8 and 0.3 of the total count at 5° and 25°C.

Cladosporium was isolated only at 5° and 25°C, and represented 100% and 83.3% of the samples constituting 17.6 and 7.1 of the total count of fungi. C. cladosporioids was of high occurrence at 5°C and moderate occurrence at 25°C, while C. herbarum was of high occurrence at both temperatures.

Cochliobolus appeared only at 25°C, represented in 36.7% of the samples constituting 1.5% of the total count. C. lunatus was of moderate occurrence, while C. sativus was of low occurrence.

Humicola appeared at 25° and 45°C represented 30 and 43.3% of the samples, constituting 0.9 and 1.5% of the total count of fungi. H. grisea Traaen was of moderate occurrence at 25°C only, while H. grisea var thermoidea (Scytalidium thermophilum) was of moderate occurrence only at 45°C.

Emericella nidulans (Aspergillus nidulellus) was isolated at 25° and 45°C and represented 43.3 and 53.3% of the samples constituting 1.5 and 3.1% of the total fungi. E. nidulans var. Lata was of moderate occurrence at 25 and 45°C and E. quadrilineata occurred low at both temperatures.

Mucor was isolated at 5° and 25°C, represented 93 and 86.7% of the samples constituting 7.3 and 6.8% of the total fungi. *M. circinelloides* was of moderate occurrence and *M. racemosus* was of high occurrence at both temperatures.

Myrothecium was isolated only at 25°C, represented 73.3% of the samples constituting 5.8 of the total fungi. M. roridum was of high occurrence, while M. verrucaria was of low occurrence.

Stachybotrys was isolated at 5° and 25°C, represented 100% and 43.3% of the samples constituting 14.9 and 1.3% of the total count of fungi. S. chartarum was of high

occurrence at 5°C and moderate occurrence at 25°C, while S. elegans was of high occurrence only at 5°C.

Trichoderma appeared only at 25°C and represented 86.7% of the samples constituting 8.5% of the total fungi. This genus consisted of two species *T. harzianum* and *T. koningii* which were of high occurrence. *Trichoderma* species were often cited as high cellulose-decomposers (Moharram *et al.*, 1995; Tengerdy and Szakacs, 2003; Zayed and Abdel-Motaal, 2005).

The other genera that appeared in this study represented one species. Botrytis cinerea, Oidiodendron griseum and Pestalotia sp. appeared only at 5°C. Botrytrichum piluliferum, Colletotrichum dematium, Gliocladium catenulatum, Phoma herburum, Scopulariopsis brevicaulis, Trichothecium roseum and Verticillium catenulatum appeared only at 25°C. Malbranchea sulfurea, Rhizomucor pusillus and Talaromyces thermophilus appeared only at 45°C. Epicoccum nigrum, Nigrospora sphaerica and Rhizopus stolonifer appeared at 5° and 25°C. No species appeared at the three incubation temperatures, 5°, 25° and 45°C. Most of these fungi isolated on rice straw during this study were isolated with different frequency of occurrence from various hemicellulosic and cellulosic materials in Egypt (Moubasher and Mazan, 1990; Moubasher, 1993; Abdel-Sater and El-Said, 2001) as well as other parts of the world (Caldwell, 1973, Bisaria and Ghose, 1981; Maheshwari et al., 2000).

References

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 J. Scient. Res.* 8: 153-168.

Abdel-Hafez, S. I. I. 1982. Cellulose-decomposing fungi of desert soil in Saudi Arabia. Mycopathologia 78: 73-78.

_____, Moubasher, A. H. and Abdel-Fattah, H. M. 1978. Cellulose-decomposing fungi of salt marshes in Egypt. *Folia Microbiol.* 23: 37-44.

and Abdel-Kader, M. I. A. 1980. Cellulose-decomposing fungi of barley grains in Egypt. *Mycopathologia* **68**: 143-147.

Abdel-Kader, M. I. A., Abdel-Hafez, A. I. I. and Abdel-Hafez, S.
I. I. 1983. Composition of the fungal flora of Syrian soils. II Cellulose-decomposing fungi. *Mycopathologia* 81: 167-171.

Abdel-Sater, M. A. and El-Said, A. H. M. 2001. Xylan-decomposing fungi and xylanolytic activity in agricultural and industrial wastes. *Int. Biodet. Biodegrad.* 47: 15-21.

Arai, T., Takaya, T., Ito, Y., Hayakawa, K., Tshima, S., Shibuya, C., Nomura, M., Yoshimi, N., Shibayama, C. and Yasuda, Y. 1998. Bronchial asthma induced by rice. *Int. Med.* **37**: 98-101.

Banerjee, S., Archana, A. and Satyanarayana, T. 1995. Xylanolytic activity and xylan utilization by thermophilic molds. *Folia Microbiol.* **40**: 279-282.

Bisaria, V. S. and Ghose, T. K. 1981. Biodegradation of cellulosic materials: Substrates, microorganisms, enzymes and products. *Enz. Microb. Technol.* 3: 90-104.

Booth, C. 1977. Fusarium. CMI, Kew, Surrey, England.

Caldwell, R. 1973. Observations on the fungal flora of decompos-

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ing beech litter in soil. Trans. Britch. Mycol. Soc. 4: 249-261.

- Cooney, D. G. and Emerson, R. 1964. Thermophilic fungi. W.H. Freman and Company. San Francisco and London.
- Coronel, L. M., Joson, L. M. and Mesina, O. G. 1991. Isolation and screening of thermophilic fungi for cellulose production. *Philippine J. Sci.* **120**: 379-389.
- Domsch, K. H., Gams, W. and Anderson, T. H. 1980. Compendium of soil fungi. Academic Press, London.
- Duncan, D. B. 1955. Multiple range and multiple (F) test. *Biometrics* 11: 1-45.
- Dytham, C. 1999. Choosing and using statistics: A biologist's guide. Blackwell Science Ltd., London, UK. p147.
- Ellis, M. B. 1971. Dematiaceous hyphomycetes. CMI, Kew, Surrey, England.
- El-Dohlob, S. M., Friend, J. and Sherief, A. A. 1985. Xylan decomposing fungi in Egyptian soil. Proceedings of the Egyptian soil. *Proc. Egypt. Bot. Soc.* (Ismallia Conference) 4: 477-487.
- El-Kady, I. A., Abdel-Hafez, S. I. I. and Moubasher, M. H. 1981. Survey of cellulose-decomposing fungi of wheat straw in Egypt. *Mycopathologia* **76**: 59-64.
- El-Nawawy, A. S., 1972. Single-cell protein from Egyptian raw materials. *Agr. Res. Rev.* **50**: 129-137.
- Gams, W. Hoekstra, E. S. and Aptroot, A. 1998. CBS course of mycology, 4th ed. Printed by Ponsen and Looyen BV, Wageningen, the Netherlands.
- Harper, S. H. T. and Lynch, J. M. 1982a. The kinetics of straw decomposition in relation to its potential to produce the phytotoxin acetic acid. J. Soil Sci. 32: 627-637.
- Helal, G. A. 1993. Halotolerant and Halophilic fungi in salt marshes of Egyptian soil. Egypt. J. Appl. Sci. 8: 205-225.
- . 2005. Bioconversion of straw into improved fodder: Mycoprotein production and cellulolytic activity of rice straw decomposing fungi. Mycobiology 33: 90-96.
- House, G. T. and Stinner, R. F. 1987. Decomposition of plant residues into tillage agroecosystems. Influence of litter on mesh size and soil arthropods. *Pedobiologia* **30**: 351-360.
- Hudson, H. J. 1972. Fungal saprophytism studies in biology No. 32. Edward Arnold, London.
- Jackson, W. L. 1962. Soil chemical analysis. Constable and Co. Ltd., London.
- Johnson, L. F., Curl, E. A., Bond, J. H. and Fribourg, H. A. 1959. Methods for studying soil micoflora. Plant disease relationships, Burgess Pub. Co., Minneapolis.
- Kitch, M. A. and Pitt, J. I. 1992. A laboratory guide to the commen *Aspergillus* species and their teleomorphs. CSIRO, Sydney.
- Kubicek, C. P. and Harman, G. E. 1998. *Trichoderma* and *Gliocladium*. Taylor and Francis Ltd, London and Bristol.
- Maheshwari, R., Bharadwaj, G. and Bhat, M. K. 2000. Thermophilic fungi: Their physiology and enzymes. *Microb. Molecul. Biol. Rev.* **64**: 461-488.
- Mazen, M. B., Moubasher, A. H. and Abdel-Hafez, A. I. I. 1980. Some ecological studies on Jordanian soil fungi. II Cellulose-decomposing fungi. *Naturalia Monspeliensia, Serie Bot. Fasc.* 40: 1-12.
- Menzies, J. D. 1957. A dipper technique for serial dilution of soil for microbial analysis. Soil Sci. Soc. Am. Proc. 21: 660.
- Moharram, A. M., Abdel-Hafez, S. I. I. and Abdel-Sater, M. A. 1995. Cellulolytic activity of fungi isolated from different substrates from the New Vally Governorate, Egypt. Abhath al-

- Yarmouk. Pure Sci. Eng. 4: 139-152.
- Morais, M. H., Romas, A. C. and Oliveira, J. S. 1999. Culture of Lentinus edodes ("Shiitake"). Silva-Lusitana 7: 153-171.
- Moubasher, A. H. 1963. Selective effects of fumigation with carbon disulphide on the soil fungus flora. *Trans. Brit. Mycol. Soc.* **46**: 338-344.
- _____ 1993. Soil fungi in Qatar and other Arab Countries. The Centre for Scientific and Applied Research, Doha, Qatar.
- , Abdel-Hafez, S. I. I. and El-Maghraby, O. M. O. 1985. Studies on soil mycoflora of Wadi Bir-El-Ain, Eastern Desert, Egypt. *Cryptogamie Mycologie* **6**: 129-143.
- and Mazen, M. B. 1990. Competitive colonization of buried cellulose film by soil fungi in Egypt. Bulletin of the Faculty of Science, Assiut University 19: 165-172.
- Mouchacca, J. 1997. Thermophilic fungi: Biodiversity and taxonomic status. *Cryptogamie, Mycol.* **18**: 19-69.
- 2000a. Thermotolerant fungi erroneously reported in applied research work as possessing thermophilic attributes. *World J. Microbiol. Biotech.* **16**: 869-880.
- _____. 2000b. Thermophilic fungi and applied research: a synopsis of name changes and synonymies. *World J. Microbiol. Biotech.* **16**: 881-888.
- Piper, C. S. 1947. Soil and plant analysis. Adelaide Univ.
- Pitt, J. I. 1979. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Academic Press, London and New York.
- _____. 1986. Alaboratory guide to common *Penicillium* species.CSIRO, Sydney.
- Rai, J. P., Sinha, A. and Govil, S. R. 2001. Litter decomposing mycoflora of rice straw. Crop. Res. 21: 335-340.
- Raper, K. B. and Fennell, D. I. 1977. The genus *Aspergillus*. Robert E.K. Publish. Co., Huntington, New York.
- and Thom, C. 1968. A manual of the *Penicillium*. Hafner Publish, Co., New York.
- Richards, L. A. 1954. Diagnosis and improvement of saline and alkali soils. U.S. Dept. Agric., Handbook, No. 60.
- Rifai, M. A. 1969. A revision on the genus *Trichoderma*. *Mycological papers* 116: 1-52.
- Samar, S., Malik, R. K., Mangat, R., Singh, S. and Ram, M. 1999. Effect of rice straw burning on the efficacy of the herbicides in wheat (*Triticum aestivum*). *Ind. J. Agronomy* 44: 361-366
- Satyanarayana, T., Jain, S. and Johri, B. N. 1988. Cellulases and xylanases of thermophilic moulds. Pp. 24-60. *In:* Agnihotri, V. P., Sarbhoy, A. K. and Kumar, D. Eds. Perspectives in Mycology and Plant Pathology, Malhotra Publ. House, New Delhi.
- Smith, N. R. and Dawson, V. T. 1944. The bacteriostatic action of rose Bengal in media used for the plate count of soil fungi. *Soil Sci.* **58**: 467-471.
- Snedecor, G. W. and Cochran, W. G. 1982. Statistical methods. 6th edition. Blackwell Science Ltd., London, UK. pp 147.
- Soytong, K. 1991. Isolation of soil fungi and screening for their cellulose degradation properties. *Kaen Kaset Khon Kaen Agricultural J.* 19: 218-225.
- Srinivasan, V. R. 1979. Production of single-cell protein from cellulose. Pp 132-137. *In* Bioconversion of organic residues for rural communities. Louisiana State Univ., Baton Rouge, Louisiana, USA.
- Tengerdy, R. P. and Szakacs, G. 2003. Bioconversion of lignocellulose in solid substrate fermentation. *Bioch. Eng. J.* 13: 169-170

- Torigoe, K., Hasegawa, S., Numata, O., Yazaki, S., Matsumaga, M., Boku, N., Hiura, M. and Ino, H. 2000. Influence of emission from rice straw burning on bronchial asthma in children. *Pediatr. Int.* **42**: 143-150.
- Watson, R. D. 1960. Soil washing improves the value of the soil dilution and the plate count method of estimating populations of soil fungi. *Phytopathol.* **50**: 792-794.
- Wise, H. D. and Schaefer, M. 1994. Decomposition of leaf litter in a mull beech forest: Comparison between canopy and herbaceous species. *Pedobiologia* **38**: 269-288.
- Yananobe, T., Mitsuishi, Y. and Takasaki, Y. 1994. Method for production of cellulolytic enzymes and method for saccharifications of cellulosic materials. Agency of Industrial Science Technology, Ministry of International Trade Industry United -States - Patent. US, 4956291.
- Zayed, G and Abdel-Motaal, H. 2005. Bio-active composts from rice straw enriched with rock phosphate and their effect on the phosphorus nutrition and microbial community in rhizosphere of cowpea. *Biores. Technol.* **96**: 929-935.