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Mycobiota Variation in Stored Rice Straw and its Cellulolytic Profile

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Abstract: Rice Straw (RS) one of most important agrowaste worldwide. Variation in mycobiota inhabiting long stored RS and its cellulolytic profile were studied. The highest number of fungi (23 species) was recovered from 1st storage period (1-3 year). *Alternaria alternata*, *Aspergillus* sp., *Cladosporium herbarum*, *Fusarium incarnatum*, *Geotrichum candidum*, *Penicillium* sp., *Stemphylium lycopersici* and *Ulocladium atrum* are the most frequent genera. Among 21 fungal species recovered in the 2nd period (3-5 year), *Cladosporium herbarum*, *Fusarium incarnatum*, *Stemphylium lycopersici* and *Ulocladium atrum* recorded 100% frequency, whereas *Ulocladium atrum*, *Veticillium lecanii*, *Stemphylium lycopersici* and *Penicillium* sp., were the most frequent species in the 3rd period (>5 years). Regarding the pathogenic fungal isolates, *Nigrospora oryzae* was the most frequent with high intensity in all samples of the three storage periods, whereas *Alternaria padwikii* reached the highest frequency and intensity in the 1st period and absent the 2nd and 3rd ones. The isolated fungal species showed a high production of cellulases comparing to previous studies with positive and significant correlation between FPase from one side and CMCase ($r = 0.634$, $p \leq 0.05$) and β-glucosidase ($r = 0.775$, $p \leq 0.05$) from the other side.

Key words: Mycobiota, rice straw borne fungi, cellulose, correlation, storage time

INTRODUCTION

Rice Straw (RS) is a renewable lignocellulosic biomass, with global production of 600 to 900 million tons year^{-1} (Karimi *et al*., 2006). It is one of the most abundant lignocellulosic waste materials in the world. According to the 'United Nations' estimation, by the year 2020, world population will have swollen to around 8 billion, of whom 5 billion will be rice consumers; the world's rice harvest must increase to 840 million metric tons year^{-1} to meet the demand. As a result, more rice straw will be produced. If it is not being used alternatively, it will create environmental problems.

In Egypt and other countries, RS stored for domestic uses for several years, left in the paddy field and discharged by landfill and open burning. The chemical composition of RS is cellulose (32-47%), hemicellulose (19-27%) and lignin (5-24%) (Saha, 2003; Kim *et al*., 2010). Hemicellulose is made up of pentose sugars, of which xylose is the most abundant (14.8-20.2%) (Roberto *et al*., 2003). These nutrients with seasonal change in temperature and humidity in tropical and subtropical countries make the RS as fungal incubator for both saprophytic and pathogenic fungi. Most of saprophytic colonizing fungal species are celulolytic which have a biotechnological potential in various industries including food, animal feed, brewing and wine making, agriculture, biomass refining, pulp and paper, textile and laundry (Singh *et al*., 2007). Where parasitic species can be infectious for the 2nd or other crop season. This study aimed to study the mycobiota associated with one of the most abundant agrowaste in the world and dealing with variation of saprophytic and pathogenic fungal species in stored and deteriorated RS and their potentiality in cellulase production.

MATERIALS AND METHODS

Isolation of fungi from rice straw: Thirty rice straw samples of different local rice cultivars from fields and storages in Dakhlia province in Egypt during 2010-2012 were used in this study. The samples represented

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different periods of rice age (1-3, 3-5 and over 5 years age) 10 samples for each. Each sample was homogenized well, cut in small pieces (0.5-1.0 cm long), 100 pieces were placed in sterile Petri plates (10 straws plate^{-1}) lined with sterile filter papers wetted with sterile H_2O. The plates incubated at 23±2°C for 7 days and exposed to cool white fluorescent light (12 h light/dark cycle). The growing fungal clonies were examined using stereo and compound microscopes to help identifying the retrieved fungi. Hyphal-tip and single spore isolation techniques was used to obtain pure cultures which maintained on Potato Dextrose Agar (PDA) slants for identification and further studies.

Identification of fungal isolates: For determination of morphological structures, portions of fungal growth were mounted in lacto-phenol cotton blue stain on clean slides, prepared slide was examined under a light microscope using the 40 and 100X objectives for vegetative mycelium; septation, diameters, conidiophores (sporangiophores) and the reproductive structures: Conidia, sporangiospores etc. Fungal colonies were examined under the 10×(low power) objective of the microscope. The colonial characteristics of size, texture and color of the colony were investigated. The fungal genera and species were identified according to their cultural properties, morphological and microscopical characteristics as described by Gams *et al.* (1998), Kubicek and Harman (1998), Moubasher (1993), Klich and Pitt (1992), Pitt (1979, 1986), Domsch *et al.* (1980), Booth (1977), Raper and Fennell (1977), Ellis (1971), Rifai (1969), Raper *et al.* (1968) and Cooney and Emerson (1964).

Solid-state fermentation growth conditions for testing fungal cellulase: In 100 mL glass flask, 1 g of dried RS moisten with 1 mL of basal salt solution contained (g L^{-1}): KH_2PO_4, 15; $(NH_4)_2SO_4$, 5; $MgSO_4.7H_2O$, 0.6; $ZnSO_4.7H_2O$, 0.14; $MnSO_4.6H_2O$, 0.16; $CaCl_2.6H_2O$, 0.37 and peptone, 2. (pH 4.8), autoclaved at 120°C for 20 min. Then inoculated with 0.5 mL of fungal spore suspension (10^6 spore mL^{-1}) of the tested species and incubated at 28±2°C for 10 days.

Elution of cellulase enzymes: Elution of cellulase enzymes were carried out by distilled water (1:10 w/v) in the presence of 2-3 drops of Berj 35. After shaking at 200 rpm for 30 min, the supernatant was separated by filtration and used for cellulases assay.

Assay of cellulases: Assay of filter paperase activity (FPase) estimates the overall cellulolytic activity. It was assayed by a modification of the method of Ghose (1987), incubating 1 mL of supernatant of culture with 50 mg Whatman No. 1 filter paper (1×6 cm) in 1 mL of 0.2 mole acetate buffer (pH 4.8) at 50°C for 60 min. One unit of FPase activity corresponding to 1 μmole of glucose equivalent released min^{-1} under the experimental assay conditions.

Carboxymethyl cellulase (1.4-β-D-glucan glucanohydrolase EC 3.2.1.4) (CMCase) was assayed using a mixture of 0.5 mL of substrate (0.5 w/v carboxymethyl cellulose, sigma) in 0.2 M acetate buffer, pH 4.8 plus 0.5 mL of enzyme, the mixture was incubated for 30 min at 50°C. One unit of CMCase activity is the amount of enzyme required to release reducing sugars equivalent to 1 μmole glucose min^{-1} under the above experimental conditions.

Beta-glucosidase (cellobiase or β D-glucoside glucohydrolase EC 3.2.1.21) activity against cellobiose was determined in a mixture of 0.1 mL of culture supernatant and 0.5 mL of cellobiose in 0.2 mole acetate buffer (pH 4.8). The reaction mixture was incubated at 50°C for 30 min. One β-glucosidase activity unit is equivalent to 1 μmole of glucose min^{-1} under the above experimental conditions.

The released reducing sugars of the 3 enzymes were measured by glucose oxidase Kit using glucose as standard (Spinreact Company, Spain).

Statistical analysis: The statistical analysis software CoStat v6.4 was used, by which all data were statistically analyzed by analysis of variance test and post comparison was carried out based on Duncan's new multiple range test. Simple correlation coefficient (r) was performed to examine the relationships between individual factors. The values of probability ($p \leq 0.05$) were considered statistically significant.

RESULTS

Generally, RS is gifted with diverse fungal species, in which, 30 species belonging to 22 genera of fungi were obtained from the collected RS samples at 3 time periods and identified based on their morphology (Table 1). The wide diverse of various isolated fungal species was classified into 2 groups, saprophytic (22 species belonging to 17 genera) and plant parasitic or pathogenic (8 species belonging 8 genera). Most of the genera were represented by 1 species except *Aspergillus* which represented by 6 species and *Alternaria* by 2 species. The highest number of fungal species was recovered in the time range of 1-3 year (23 fungal species) as compared to 3-5 year (21 fungal species) and >5 years (16 fungal species) time periods. Considerable differences in the incidence and frequency of the total recorded straw brone

Table 1: Frequency and intensity percentages of different fungi in stored RS sample

Fungus	1st period (1-3 year)		2nd period (3-5 year)		3rd period (>5 year)	
	Frequency (%)[1]	Intensity (%)[2]	Frequency (%)	Intensity (%)	Frequency (%)	Intensity (%)
Saprophytic fungi						
Alternaria alternata	100 (2-28.5)[3]	15.1^{c-g}	80 (0-4)	2.3^{i-m}	60 (0-3.5)	1.1^{lm}
Aspergillus flavus	40 (0-1)	0.4^{m}	0.0	0.0^{m}	0.0	0.0^{m}
Aspergillus glaucus	60 (0-62)	18.0^{c-e}	0.0	0.0^{m}	0.0	0.0^{m}
Aspergillus niger	80 (0-58)	19.0^{cd}	20 (0-4)	0.8^{lm}	0.0	0.0^{m}
Aspergillus ochraceus	60 (0-14)	4.8^{h-m}	0.0	0.0^{m}	0.0	0.0^{m}
Aspergillus tamaria	40 (0-1)	0.4^{m}	0.0	0.0^{m}	0.0	0.0^{m}
Aspergillus sp.	100 (4-28)	11.3^{d-k}	60 (0-4.8)	1.6^{j-m}	20 (0-2)	0.4^{m}
Cephalosporium acremonium	40 (1-4)	1.0^{lm}	40 (0-5)	1.4^{k-m}	0.0	0.0^{m}
Chaetomium globosum	0.0	0.0^{m}	100 (5-25)	16.5^{c-f}	60 (0-2)	0.7^{lm}
Cladosporium herbarum	100 (25-86)	64.6^{a}	100 (4-64.62)	12.1^{d-i}	60 (0-2)	0.9^{lm}
Epicoccum purpurascens	60 (0-4.2)	1.4^{k-m}	40 (0-2)	0.6^{m}	20 (0-2)	0.4^{m}
Fusarium incarnatum	100 (2-18)	8.8^{e-m}	100 (1-9)	4.0^{i-m}	20 (0-3)	0.6^{m}
Geotrichum candidum	100 (8-55)	23.8^{bc}	80 (3-27)	8.0^{f-m}	60 (0-17.1)	5.7^{g-m}
Mucor indicus	60 (0-3)	1.0^{lm}	0.0	0.0^{m}	0.0	0.0^{m}
Penicillium sp.	100 (17-38)	31.5^{b}	60 (0-3)	1.0^{lm}	80 (0-26.7)	8.9^{e-m}
Rhizopus stolonifer	0.0	0.0^{m}	20 (0-1.5)	0.3^{m}	0.0	0.0^{m}
Stachybotrys chartarum	40 (0-13)	3.1^{i-m}	100 (2-31.5)	10.7^{d-l}	60 (0-2)	0.7^{lm}
Stemphylium lycopersici	100 (0.5-25.5)	9.3^{e-m}	100 (2-15.5)	10.7^{d-l}	80 (0-13.5)	5.3^{h-m}
Trichoderma viride	0.0	0.0^{m}	40 (1-3)	0.8^{lm}	40 (0-2.5)	0.7^{lm}
Trichothecium rosum	60 (2-7)	2.2^{i-m}	0.0	0.0^{m}	0.0	0.0^{m}
Ulocladium atrum	100 (1.5-38.5)	14.5^{d-h}	100 (3-23.5)	16.0^{c-f}	80 (0-20)	7.7^{f-m}
Verticillium lecanii	0.0	0.0^{m}	80 (1-18)	5.2^{h-m}	80 (0-13.8)	6.9^{f-m}
Pathogenic fungi						
Alternaria padwikii	60 (0-17)	5.0^{h-m}	0.0	0.0^{m}	0.0	0.0^{m}
Bipolaris oryzae	60 (2-7)	11.5^{d-j}	20 (0-0.5)	1.0^{lm}	0.0	3.1^{i-m}
Curvularia lunata	0.0	0.0^{m}	40 (0-1)	0.4^{m}	0.0	0.0^{m}
Fusarium verticillioides	20 (0-1)	0.2^{m}	20 (0-4.5)	0.9^{lm}	0.0	0.0^{m}
Nigrospora oryzae	80 (0-17)	10.1^{d-m}	40 (0-4)	1.0^{lm}	60 (0-9.24)	3.2^{i-m}
Phoma sorghina	0.0	0.0^{m}	0.0	0.0^{m}	20 (0-2)	0.4^{m}
Sarocladium oryzae	40 (0-5)	1.2^{lm}	0.0	0.0^{m}	0.0	0.0^{m}
Ustilaginoidea virens	0.0	0.0^{m}	20 (0-2.2)	0.4^{m}	0.0	0.0^{m}

Intensity designated with different letter are significantly differed at $p \le 0.05$. [1]Frequency of a fungus on RS (%) = (No. of RS samples on which the fungus was detected/Total No. of investigated RS samples)×100, [2]Intensity of a fungus on RS (%) = (Mean of infection of RS samples with a fungus/Total No. of investigated RS samples)×100, [3]No. in parenthesis indicate occurrence range on the various RS samples

fungi (saprophytic and pathogenic) were observed, over the different periods. The highest incidence was recorded by *Cladosporium herbarum*, (64.6%) with an occurrence range of 25 to 86%, followed by *Penicillium* sp. (31.5%), *Geotrichum candidum* (23.8%), *Aspergillus niger* (19%), *A. glaucus* (18%), *Alternaria alternate* (15.1%) and *Ulocladium atrum* (14.5%) which were the most abundant in straw samples in the 1st period (1-3 year). High incidence of *Chetomium globosum* and *Ulocladium atrum* (16.5 and 16%, respectively) was detected in the collected samples of 3-5 year age, followed by *Cladosporium herbarum*, *Stachybotrys chartarum* and *Stemphylium lycopersici* (12.1, 10.7 and 10.7%, respectively). In >5 year of time, *Penicillium* sp., *Ulocladium atrum*, *Veticillium lecanii*, *Geotrichum candidum* and *Stemphylium lycopersici* (8.9, 7.7, 6.9, 5.7 and 5.3%, respectively) were the abundant recovered saprophytic fungi.

Concerning the saprophytic fungal isolates, in the 1st storage period (1-3 year) *Alternaria alternata*, *Aspergillus* sp., *Cladosporium herbarum*, *Fusarium incarnatum*, *Geotrichum candidum*, *Penicillium* sp., *Stemphylium lycopersici* and *Ulocladium atrum* were the most frequent genera. The intensity of the most frequent species are not correlated with its frequency as it ranged from 8.8-64.4%. In 3-5 year period some species as in the 1st period like *Cladosporium herbarum*, *Fusarium incarnatum*, *Stemphylium lycopersici* and *Ulocladium atrum* recorded 100% frequency, whereas most of Aspergillia species like *A. flavus*, *A. glaucus*, *A. ochraceus* and *A. tamaria* as well as *Trichothecium rosum* were absent in samples of this period. On the other hand, *Chaetomium globosum*, *Veticillium lecanii* and *Trichoderma viride* which totally absent in the 1st period, appeared in 100, 80 and 40%, respectively. In the 3rd period (>5 years), the intensity of the total isolates are lower than that in the 1st and 2nd period. Most of Aspergillia species as well as *Mucor* sp. and *Trichothecium rosum* absent in the samples of the 3rd period, oppositely, the most frequent species are *Ulocladium atrum*, *Veticillium lecanii*, *Stemphylium lycopersici* and *Penicillium* sp., all being represented by 80%.

Regarding the pathogenic fungal isolates, *Nigrospora oryzae* was the most frequent with high intensity in all samples of the three periods, whereas the

frequency and intensity of *Alternaria padwikii* is high in the 1st period and absent in the 2nd and 3rd ones. *Curvularia lunata* and *Ustilaginoidea virens* absent from 1st and 3rd period where they showed 40 and 20% frequency in the 2nd period, respectively. *Phoma sorghina* has different pattern than most of pathogenic species as it absent in samples of 1st and 2nd period and recorded 20% frequency in the 3rd one.

The potential of the previous survey remains unexplored, so, the tested points (RS age and fungal isolates) need to be clarified with some details, in terms of statistical separated of RS age and the different fungal isolates. This separation is to evaluate the influence and the nature of relationship within each single factor (straw age and the recovered fungi) on the diversity of the mycobiota on RS. As for the influence of age of RS (Fig. 1), the statistical analysis shows that time is greatly affecting on the incidence of the total fungal species, irrespective the various isolated fungi. However, there was a remarkable decline in the incidence of the total fungi with the prolongation of the straw age. To provide an insight on the possible relation between the straw age and the total fungal isolates, the simple correlation (r) and

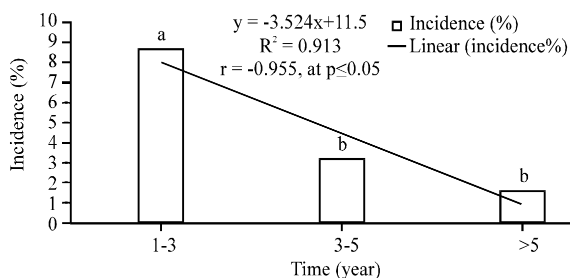

Fig. 1: Effect of time on the incidence of the total fungi (irrespective the fungal species) isolated from different RS samples. Columns designated with different letter are significantly differed at $p \leq 0.05$

regression (r^2) coefficients were calculated, wherein, r which is used to descript the qualitative nature between factors recorded -0.955 at $p \leq 0.05$. This value reflecting strong negative relation between storage time and the total fungal incidence. Unlike r, r^2 quantitatively determines the relationship between the tested factors that represents the rate of change of one variable (fungal incidence) as a function of changes in the other (straw age). Based on these rules there is a significant r^2 and its value recorded 0.913. However, the closer of r and r^2 to one, the tight relationship between the variables. Regarding the relationship between the incidence of individual fungal isolate and the total fungi, irrespective the storage age (Fig. 2), there was distinctive trend, in which the saprophytic fungi increased compared to the pathogenic fungi. Although *Cladosporium herbarum* is by far the most widely presented fungus, it is, fortunately, classified as saprophyte not as pathogen.

Cellulases activity of the isolated species: As shown in Table 2, the isolated fungal species showed a great variation in its cellulases activity, for FPase the most active isolate was *Penicillium* sp. (36.29 U g^{-1} RS) followed by *A. niger* (32.73 U g^{-1} RS). Isolates of *F. incarnatum*, *Cladosporium herbarum* and *T. viride* have a moderate activity ranged around 20 U g^{-1} RS, on the other side, very weak and no activity was showed by *Trichothecium rosum* and *Bipolaris oryzae*. The most active isolates in CMCase are *T. viride*, *Stemphylium lycopersici* and *Penicillium* sp. (177.74, 161.7 and 152.69, respectively). *Alternaria padwike*, *Epicoccum purpurascens* and *A. oryzae* have a relatively moderate activity (around 60% of the highest one). *Bipolaris oryzae* have the lowest activity in FPase. There are dramatic differences in the activity of β-glucosidase between the isolated species. The data also clarify that, *Ulocladium atrum*, *Trichothecium rosum*, *Cladosporium globosum*, *Bipolaris oryzaea* and

Table 2: Cellulases activity of the isolated fungal species

Fungus	FPase (U g^{-1} RS) (%)	CMCase (U g^{-1} RS) (%)	β-glucosidase (U g^{-1} RS) (%)
Alternaria padwike	14.20 (39.1)	122.00 (68.8)	0.13 (0.0)
Aspergillus oryzae	12.16 (33.5)	111.31 (62.2)	0.00 (0.0)
Aspergillus niger	32.73 (90.2)	101.99 (57.4)	217.59 (100)
Bipolaris oryzae	0.00 (0.0)	16.25 (9.1)	0.00 (0.0)
Chaetomium globosum	3.89 (10.7)	38.77 (21.8)	1.50 (0.0)
Cladosporium herbarum	20.81 (57.3)	109.40 (61.6)	7.30 (3.4)
Epicoccum purpurascens	12.42 (34.2)	113.60 (63.9)	14.33 (6.6)
Fusarium incarnatum	23.87 (65.8)	100.62 (56.6)	0.00 (0.0)
Penicillium sp.	36.29 (100)	152.69 (85.9)	181.97 (83.6)
Stachybotrys chartarum	4.02 (11.1)	96.80 (54.5)	11.43 (5.3)
Stemphylium lycopersici	12.29 (33.9)	161.70 (91.0)	48.99 (22.5)
Trichoderma viride	18.40 (50.7)	177.74 (100)	42.43 (19.5)
Trichothecium rosum	1.09 (3.0)	50.15 (28.2)	0.00 (0.0)
Ulocladium atrum	3.76 (10.4)	50.61 (28.5)	0.00 (0.0)

No. in parenthesis represents the percentage from the highest recorded activity for each enzyme

Table 3: Correlation coefficients between every pairs of the 3 types of cellulases and storage periods of RS

Parameter	FPase	CMCase	β-glucosidase	Frequency (1-3 year)	Intensity (1-3 year)	Frequency (3-5 year)	Intensity (3-5 year)	Frequency (>5 year)
CMCase	0.634*							
β-glucosidase	0.775*	0.374						
Frequency (1-3 year)	0.209	0.111	0.282					
Intensity (1-3 year)	0.422	0.120	0.269	0.607*				
Frequency (3-5 year)	-0.151	0.058	-0.112	0.203	0.330			
Intensity (3-5 year)	-0.362	-0.259	-0.266	0.058	0.221	0.886*		
Frequency (>5 year)	0.028	0.261	0.094	0.262	0.334	0.887*	0.738*	
Intensity (>5 year)	0.136	0.094	0.304	0.517*	0.269	0.404	0.292	0.687*

*Significant at $p \leq 0.05$

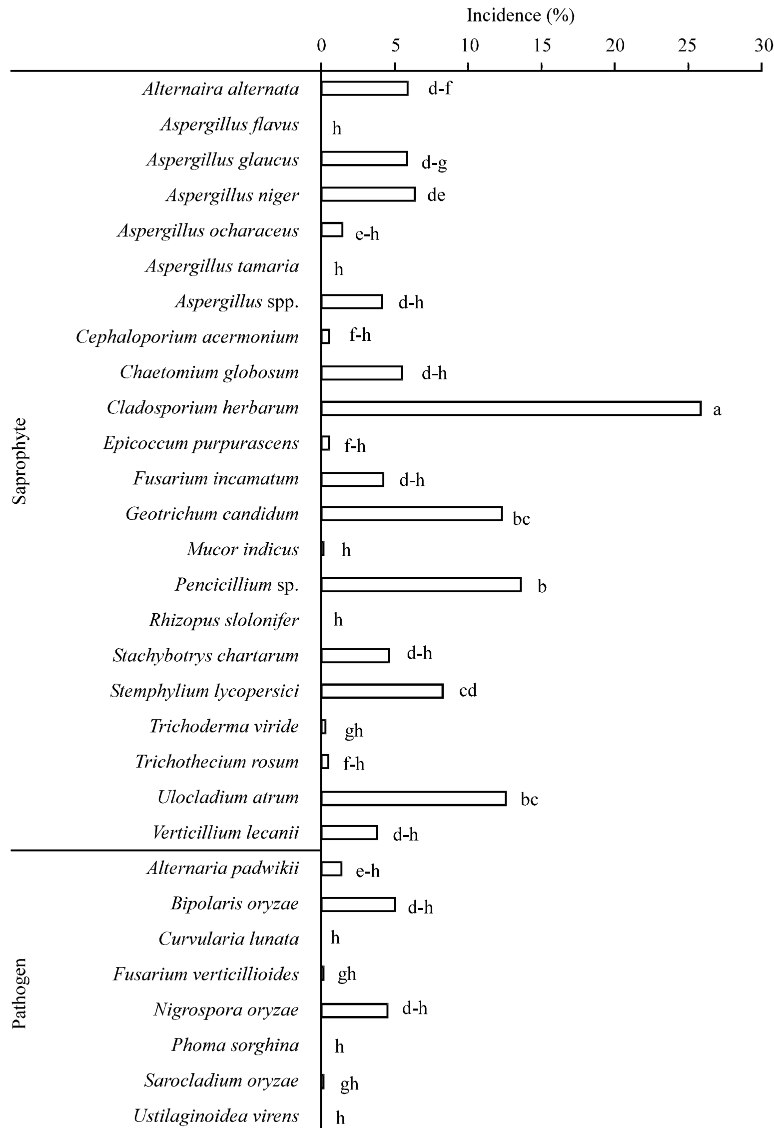

Fig. 2: Comparison among individual fungal species associated with RS samples (irrespective the time). Columns designated with different letter are significantly differed at $p \leq 0.05$

A. oryzae isolates have no activity, where, *Cladosporium herbarum* and *Stachybotrys chartarum* have very weak activity not exceed 6% of the highest one which represented by *A. niger* (217.59 U g^{-1} RS) followed by *Penicillium* sp. (181.97 U g^{-1} RS).

Data of the analysis of correlation coefficient (Table 3) revealed a positive and significant correlation between FPase from one side and CMCase ($r = 0.634$, $p \leq 0.05$) and β-glucosidase ($r = 0.775$, $p \leq 0.05$) on the other side, in spite of the positive correlation between CM Case and β-glucosidase, this correlation did not reach the level of significance ($r = 0.374$). During any of the test period, there was positive significant correlation between the fungal frequency and intensity, recording, 0.607, 0.886 and 0.687 in the 1st, 2nd and 3rd periods, respectively. In addition, there was an evidence for positive significant correlation between the frequency and Intensity of the 2nd period (3-5 year) and frequency of the 3rd period.

DISCUSSION

Storing of RS is common in many rice cultivation countries and Egypt is one of them. In this work, the long storage period revealed remarkable changes in diversity and occurrence of RS inhibiting mycobiota. For saprophytic fungi, 22 species belonging to 17 genera were isolated which is more than that isolated by Darmwal and Gaur (1991) who isolated nineteen cellulolytic fungi of nine different species belonging to seven genera on rose bengal cellulose agar medium. In the same pattern, 25 species belonging to 16 genera of filamentous fungi colonizing rice straw isolated from 11 different sites in Korea (Lee *et al*., 2011). Moreover, Shen *et al.* (1990) obtained similar isolates from 2 factories processing rice and wheat straw near Shanghai, China. The dominant fungal species isolated from RS are similar to that isolated from 287 rice seed samples of 20 cultivars representing different locations of Tamil Nadu state, India with the most predominant one *Bipolaris oryzae* that was associated with 58.89% of seed samples, followed by *Alternaria padwickii* (52.96%) (Gopalakrishnan *et al*., 2010).

The relatively high degree of diversity in our isolates may be due to our simple and representative method of isolation by putting the RS pieces over sterilized moisten filter paper make the RS itself as a source of fungi and in the same time the only nutritive source of growth which more similar to that happened in the nature as the type of substrate, water activity, temperature and air gas composition are the most important parameters affect fungal growth in post-harvest (Magan and Lacey, 1984; Magan *et al*., 2003). The most common isolated genera are *Fusarium*, *Penicillium* and *Aspergillus* which can live in different, partially overlapping ecological niches (Giorni *et al*., 2009). *Fusarium* is commonly dominant in the field, where water activity is not a limiting factor for most of the crop-growing period; it can continue its development during storage with suitable humidity (Magan and Lacey, 1984). *Aspergillus* and *Penicillium* on the contrary, is more xerophilic and could get the upper hand during storage (Marin *et al*., 1998).

Some fungal species that not noticed in the 1st period and have a high frequency and intensity in the 2nd and 3rd period like *Verticillium* which is a soil and seed-borne deuteromycete fungus (Rowe and Powelson, 2002), can survive for several years as microsclerotia (Schnathorst, 1981). *Stachybotrys chartarum* which found in considerable frequency in the 3 periods, is known as producer of a number of potent mycotoxins, including macrocyclic trichothecenes, roridin, satratoxins and isosatratoxins (Kuhn and Ghannoum, 2003; Straus, 2009). Its surviving and high intensity after more than 5 years of storage can be explained by Andersen and Nissen (2000), who suggested that the production of spores by *Stachybotrys* in slime heads prevent the spores from readily aerosolizing.

The frequency and intensity of most of parasitic fungi decreased with increasing the storage period and this can be explained by its prefer parasitic life pattern as much of them cannot grow saprophyticlly on RS. Most of pathogenic isolates are rice parasite, like *Alternaria padwickii* which causes stackburn disease, a common seed-borne fungus in different rice cultivars in Egypt as well as in most of the rice-growing countries worldwide (Mew and Gonzales, 2002; Mathur and Manandhar, 2003). The disease is not considered economic importance but it may be more severe in restricted areas of a field under disease favorable conditions. Tisdale (1922) stated that the fungus doubtless lives through the winter in the soil and old rice straw and infects the rice plants during the growing season. Rice brown spot, caused by *Bipolaris oryzae*, is one of the important diseases in the world including Egypt (Mew and Gonzales, 2002). Widespread, reported in all rice-growing countries of Asia, America and Africa (Webster and Gunnell, 1992). In Egypt, the disease comes in the 2nd rank after blast disease (El-Wahsh, 1997; Shabana *et al*., 2008). It can be a serious disease causing a considerable yield loss. Where, it affects the quality and the number of grains per panicle and reduces the kernel weight (Mew and Gonzales, 2002). *Nigrospora oryzae* is spread in all isolation periods because it is mainly saprotroph colonizing debris of different plant species and weak parasite producing grain spots of rice, sorghum and corn (Neergaard, 1977), it commonly found among fungi associated with seed of different cereals (Baszkowski and Piech, 2002). Another example of the isolated pathogens is *Phoma sorghina* that causes rice to leaf spot (Sert and Sumbul, 2005).

Studying cellulase profile using RS as a source of fungi and in the same time, the only carbon source for cellulase production, revealed enhancement in cellulase biosynthesis. As several studies have shown that cellulase biosynthesis was higher when organisms are exposed to complex substrates than grown on simple substrates (Henriksson *et al*., 2000; Wymelenberg *et al*., 2005). We used solid-state fermentation due its lower capital investment and operating expenses as well as its simplicity and closeness to the natural way of life for many microorganisms, high volumetric productivity and as an alternative in preventing environmental pollution (Chahal, 1991; Pandey, 1994). For all tested fungal isolates, cellulase levels of CMCase are always higher than the FPase and this is true for all previous studies (Kubicek, 1992; Wen *et al*., 2005) and using different substrates for fermentation (Rajoka and Malik, 1997).

Generally, when rice straw was used as a substrate for cellulases production, interesting results were observed and in comparison with previous studies, (Kang *et al*., 2004; Sherief *et al*., 2010; Lee *et al*., 2011) the fungal species isolated from stored RS showed higher activity in cellulases production when grown in RS than that isolated from other sources.

In conclusion, stored wastes with seasonal variation in nutritional and environmental conditions make it a natural microbial incubator in which we can found a good fungal isolates with special physiological potentiality than the other isolation sources.

REFERENCES

Andersen, B. and A.T. Nissen, 2000. Evaluation of media for detection of *Stachybotrys* and *Chaetomium* species associated with water-damaged buildings. Int. Biodeter. Biodegrad., 46: 111-116.

Baszkowski, J. and M. Piech, 2002. Comparison of seed-borne fungal communities of naked and husked oats and barley. Phytopath. Pol., 24: 71-74.

Booth, C., 1977. *Fusarium*: Laboratory Guide to the Identification of the Major Species. Commonwealth Mycological Institute (CMI), Kew, Surrey, UK., ISBN-13: 9780851983837, Pages: 58.

Chahal, D.S., 1991. Production of *Trichoderma reesei* Cellulase System with High Hydrolytic Potential by Solid-State Fermentation. In: Enzymes in Biomass Conversion (ACM Symposium Series, Volume 460), Leatham, G.F. and M.E. Himmel (Eds.). Chapter 9, American Chemical Society, Washington, DC., USA., ISBN-13: 9780841219953, pp: 111-122.

Cooney, D.G. and R. Emerson, 1964. Thermophilic Fungi. San Francisco Company, London.

Darmwal, N.S. and A.C. Gaur, 1991. Isolation of cellulolytic fungi for cellulase and protein production from rice straw. Zentralblatt Mikrobiologie, 146: 467-470.

Domsch, K.H., W. Gams and T.H. Anderson, 1980. Compendium of Soil Fungi. Academic Press, New York.

El-Wahsh, S.M., 1997. Studies on both brown spot and blast diseases of rice in Egypt. Ph.D. Thesis, Tanta University, Egypt.

Ellis, M.B., 1971. Dematiaceous Hyphomycetes. 1st Edn., Commonwealth Mycological Institute, Kew, Surrey, UK., pp: 608.

Gams, W., E.S. Hoekstra and A. Aptroot, 1998. CBS Course of Mycology, 4th Edn., CBS, Baarn, The Netherlands.

Ghose, T.K., 1987. Measurement of cellulase activities. Pure Applied Chem., 59: 257-268.

Giorni, P., N. Magan and P. Battilani, 2009. Environmental factors modify carbon nutritional patterns and niche overlap between *Aspergillus flavus* and *Fusarium verticillioides* strains from maize. Int. J. Food Microbial., 130: 213-218.

Gopalakrishnan, C., A. Kamalakannan and V. Valluvaparidasan, 2010. Survey of seed-borne fungi associated with rice seeds in Tamil Nadu, India. Libyan Agric. Res. Cent. J. Int., 1: 307-309.

Henriksson, G., G. Johansson and G. Pettersson, 2000. A critical review of cellobiose dehydrogenases. J. Biotechnol., 78: 93-113.

Kang, S.W., Y.S. Park, J.S. Lee, S.I. Hong and S.W. Kim, 2004. Production of cellulases and hemicellulases by *Aspergillus niger* KK2 from lignocellulosic biomass. Bioresour. Technol., 91: 153-156.

Karimi, K., G. Emtiazi and M.J. Taherzadeh, 2006. Production of ethanol and mycelial biomass from rice straw hemicellulose hydrolyzate by *Mucor indicus*. Process. Biochem., 41: 653-658.

Kim, M.J., H. Lee, Y.S. Choi, G.H. Kim and N.Y. Huh *et al*., 2010. Diversity of fungi in creosote-treated crosstie wastes and their resistance to polycyclic aromatic hydrocarbons. Antonie van Leeuwenhoek, 97: 377-387.

Klich, M.A. and J.I. Pitt, 1992. A Laboratory Guide to the Common *Aspergillus* Species and their Teleomorphs. CSIRO Division of Food Processing, North Ryde, Australia, ISBN: 9780643048669.

Kubicek, C.P. and G.E. Harman, 1998. Trichoderma and Gliocladium, Volume 1: Basic Biology, Taxonomy and Genetics. Taylor and Francis Ltd., London, UK., ISBN: 0748405720, Pages: 278.

Kubicek, C.P., 1992. The Cellulase Proteins of *Trichoderma reesei*: Structure, Multiplicity, Mode of Action and Regulation of Formation. In: Enzymes and Products from Bacteria Fungi and Plant Cells, Coolbear, T. (Ed.). Vol. 45, Springer-Verlag, Berlin, Germany, ISBN: 978-3-540-55106-5, pp: 1-27.

Kuhn, D.M. and M.A. Ghannoum, 2003. Indoor mold, toxigenic fungiand *Stachybotrys chartarum*: Infectious disease perspective. Clin. Microbiol. Rev., 16: 144-172.

Lee, S., Y. Jang, Y.M. Lee, J. Lee, H. Lee, G.H. Kim and J.J. Kim, 2011. Rice straw-decomposing fungi and their cellulolytic and xylanolytic enzymes. J. Microbiol. Biotechnol., 21: 1322-1329.

Magan, N. and J. Lacey, 1984. Effect of temperature and pH on water relations of field and storage fungi. Tran. Br. Mycol. Soc., 82: 71-81.

Magan, N., R. Hope, V. Cairns and D. Aldred, 2003. Post-harvest fungal ecology: Impact of fungal growth and mycotoxin accumulation in stored grain. Eur. J. Plant Pathol., 109: 723-730.

Marin, S., V. Sanchis, A.J. Ramos, I. Vinas and N. Magan, 1998. Environmental factors, *in vitro* interactions and niche overlap between *Fusarium moniliforme*, *F. proliferatum* and *F. graminearum*, *Aspergillus* and *Penicillium* species from maize grain. Mycol. Res., 102: 831-837.

Mathur, S.B. and H.K. Manandhar, 2003. Fungi in Seeds: Recorded at the Danish Government Institute of Seed Pathology for Developing Countries. 1st Edn., Danish Government Institute of Seed Pathology for Developing Countries, Copenhagen, Denmark, ISBN-13: 9788798983323, Pages: 825.

Mew, T.W. and P. Gonzales, 2002. A Handbook of Rice Seedborne Fungi. International Rice Research Institute, USA., ISBN: 9789712201745, Pages: 83.

Moubasher, A.H., 1993. Soil Fungi in Qatar and other Arab Countries. 1st Edn., Center of Scientific and Applied Research, University of Qatar, Doha, Qatar, ISBN-13: 9992121025, Pages: 566.

Neergaard, P., 1977. Seed Pathology. Vol. 1, The Macmillan Press Ltd., London and Basingstoke.

Pandey, A., 1994. Solid-State Fermentation: An Overview. In: Solid-State Fermentation, Pandey, A. (Ed.). Wiley Eastern, New Delhi, India.

Pitt, J.I., 1979. The Genus *Penicillium* and its Teleomorphic States: *Eupenicillium* and *Talaromyces*. Academic Press, London, UK., ISBN-13: 9780125577502, Pages: 634.

Pitt, J.I., 1986. A Laboratory Guide to common *Penicillium* Species. CSIRO, Division of Food Research, Australia.

Rajoka, M.I. and K.A. Malik, 1997. Cellulase production by *Cellulomonas biazotea* cultured in media containing different cellulosic substrates. Bioresour. Technol., 59: 21-27.

Raper, K.B., C. Thom and D.I. Fennell, 1968. A Manual of the Penicillia. Hafner Publishing Co. Inc., New York, USA., ISBN-13: 978-0028508306, Pages: 876.

Raper, K.B. and D.I. Fennell, 1977. The Genus *Aspergillus*. Williams and Wilkins, Huntington, New York.

Rifai, M.A., 1969. A revision of the genus *Trichoderma*. Mycol. Pap., 116: 1-56.

Roberto, I.C., S.I. Mussatto and R.C.L.B. Rodrigues, 2003. Dilute-acid hydrolysis for optimization of xylose recovery from rice straw in a semi-pilot reactor. Ind. Crops Prod., 17: 171-176.

Rowe, R.C. and D.L. Powelson, 2002. Potato early dying: Management challenges in a changing production environment. Plant Dis., 86: 1184-1193.

Saha, B.C., 2003. Hemicellulose bioconversion. J. Ind. Microbiol. Biotechnol., 30: 279-291.

Schnathorst, W.C., 1981. Life Cycle and Epidemiology of *Verticillium*. In: Fungal Wilt Diseases of Plants, Mace, M.E., A.A. Bell and C.H. Beckman (Eds.). Academic Press, New York, pp: 81-111.

Sert, H.B. and H. Sumbul, 2005. First report of leaf spot caused by *Phoma sorghina* on *Trifolium campestre* in Turkey. Plant Pathol., 54: 249-249.

Shabana, Y.M., G.M. Abdel-Fattah, A.E. Ismail and Y.M. Rashad, 2008. Control of brown spot pathogen of rice (*Bipolaris oryzae*) using some phenolic antioxidants. Braz. J. Microbiol., 39: 438-444.

Shen, Y.E., W.G. Sorenson, D.M. Lewis and S.A. Olenchock, 1990. Microbiological analyses and inflammatory effects of settled dusts from rice and hay. Biomed. Environ. Sci., 3: 353-363.

Sherief, A.A., A.B. El-Tanash and N. Atia, 2010. Cellulase production by *Aspergillus fumigatus* grown on mixed substrate of rice straw and wheat bran. Res. J. Microbiol., 5: 199-211.

Singh, A., R.C. Kuhad and O.P. Ward, 2007. Industrial Application of Microbial Cellulases. In: Lignocellulose Biotechnologgy: Future Prospects, Kuhad, R.C. and A. Singh (Eds.). I.K. International Publishing House, New Delhi, India, pp: 345-358.

Straus, D.C., 2009. Molds, mycotoxins and sick building syndrome. Toxicol. Ind. Health, 25: 617-635.

Tisdale, W.H., 1922. Seedling blight and stack-burn of rice and the hot-water seed treatment. Bulletin of the United States Department of Agriculture No. 1116, USDA, Washington, DC., USA., November 13, 1922, pp: 1-11.

Webster, R.K. and P.S. Gunnell, 1992. Compendium of Rice Diseases. The American Phytopathological Society, St. Paul, MN., USA., ISBN-13: 9780890541265, Pages: 62.

Wen, Z., W. Liao and S. Chen, 2005. Production of cellulase by *Trichoderma reesei* from dairy manure. Bioresour. Technol., 96: 491-499.

Wymelenberg, A.V., G. Sabat, D. Martinez, A.S. Rajangam and T.T. Teeri *et al*., 2005. The *Phanerochaete chrysosporium* secretome: Database predictions and initial mass spectrometry peptide identifications in cellulose-grown medium. J. Biotechnol., 118: 17-34.