



Glucophilic fungi of sugarcane bagasse in Egypt

Mohamed S. Youssef^{1*}, Osman M. El-Maghraby¹ Nashwa A. H. Fetyan², Zienab A. Hammam³

¹ Botany and Microbiology Department, Faculty of Science, Sohag University, Sohag, Egypt.

² Microbiology Department, Soil, Water & Environment Research Institute, Agricultural Research Center, Giza, Egypt

³ Microbiology Department, Soil, Water & Environment Research Institute, Agricultural Research Center, Shandaweel Research Station, Sohag, Egypt.

Citation: Youssef, M. S., El-Maghraby, O. M., Nashwa A. H., Zienab A. H., (2021). *Glucophilic fungi of sugarcane bagasse in Egypt*. *Journal of Environmental Studies*, Vol. 25(1): 39-50. doi: [10.21608/jesj.2021.228288](https://doi.org/10.21608/jesj.2021.228288)

Article Information

Received 28 Mar. 2021,

Revised 15 May 2021,

Accepted 1 June 2021.

Published online

30 June 2021

Abstract: Thirty sugarcane bagasse samples were collected from different sugarcane juice mills in Sohag Governorate, Upper Egypt. The moisture content of tested samples was fluctuated between 32.58 - 70.59%. Total carbohydrates were estimated, and the richest sample was No. 21 with 535.10 mg/g dry weight, whereas the lowest sample was No. 3 with 508.131 mg/g dry weight. Soluble sugars of different samples were determined using spectrophotometer at 620 nm. The soluble sugars content ranged between 120 - 160 mg/g dry weight. Dilution- (8 genera, 27 species and 4 varieties) and direct- (8 genera, 25 species and 4 varieties) plate methods on glucose-Czapek's agar medium at 28 ± 2 oC were used for isolation of bagasse glucophilic fungi. It was found that the total fungal populations in case of dilution-plate method (69720 cfu/g) was so high than those of direct-plate method (828 cfu/ 20 bagasse segments). *Aspergillus* was appeared in high population and was parallel to those of total fungal counts (51700 cfu/g, 74.15 % of total count, in 29 samples with 96.7% of the samples in dilution method and 731 cfu/ 20 segments, 88.3% of total count, in 30 samples with 100% of the tested samples using direct-plate method.

Keywords: sugarcane bagasse, fungi, total and soluble carbohydrates.

Introduction

Agricultural waste is one of the major environmental pollutants; their biological recycling or biotechnological conversion is not only a remedy for environmental problems but also the source of suitable microbial by-products like food, fuel, and chemicals (Milala *et al.*, 2005). Egypt generates massive amounts of agricultural solid wastes every year, where they were estimated about 33 million tons in 2016 according to (the report of Ministry of Environment in 2016). These wastes are characterized as coarse plant by-products and big size, chemically low in protein and fat contents. Also, it is high in lignin and cellulose contents, whereas three major structural polymers combined to make up lignocellulosic wastes are called cellulose, hemicellulose, and lignin (Rajoka, 2005).

Agro-industrial wastes are interesting substrates for fermentative processes since they are easily available, rich in carbon and often represent a problem of disposal (Gassara *et al.*, 2010). One of the largest cellulosic agro-industrial by-products is sugarcane bagasse.

It is a lignocellulosic residue (by-product) of the sugar industry and is almost completely used by the sugar factories theme selves as fuel for the boilers.

In the recent years, there has been an increasing trend towards more efficient utilization of agro-industrial residues, including sugarcane bagasse. Several processes and products have been reported that utilize sugarcane bagasse as a raw material. These include electricity generation, pulp, paper production, and products based on fermentation.

Bagasse consists of approximately 50% cellulose and 25% each of hemicellulose and lignin. Chemically, bagasse contains about 50% α - cellulose, 30% pentosans, and 2.4% ash. Because of its low ash content, bagasse offers numerous advantages in comparison to other crop residues such as rice straw and wheat straw, which have 17.5% and 11.0%, respectively, ash contents, for usage in bioconversion processes using microbial cultures (Pandey *et al.*, 2000).

Sugarcane industry is one of the oldest in Egypt. Cane plantations are concentrated in Upper Egypt

* Corresponding author E-mail: youssefm2006@yahoo.com

specifically in Minia, Sohag, Qena, Luxor, and Aswan. Sugarcane is produced and harvested in Egypt for four purposes: production of cane sugar in the large-scale mills, production of black honey in smaller scale factories, use as seed for subsequent plantings and squeezed in juice-shops to make cane juice. The total amount of cane cultivated in Upper Egypt is about 16 million tons per year. The percentage of residues generated during the sugar production process is as follows; 30% bagasse, 4% molasses and 3.5% filter mud (cachaza).

These percentages amount to an annual production of about 3 million tons of bagasse, 370 thousand tons of molasses and 316 thousand tons of filter mud (Hamada 2011).

Materials and method

I. Collection of samples

Thirty samples of bagasse were collected from different sugarcane juice mills in different places in Sohag Governorate. Each sample was about 3kg. Each Sample was kept separately inside clean sealed sterilized bags, transferred directly to the laboratory, and kept at 4°C. Moisture content, total carbohydrates, soluble and insoluble sugars were estimated for each sample. Survey, isolation, and identification of glucophilic fungi were performed for each sample tested.

II. Determination of moisture content

The moisture content of bagasse was determined by oven dry method as employed by Van Reeuwijk (2002). Twenty grams of each bagasse sample (W_1) in a ceramic crucible were dried in an oven at 105 OC for 24 hrs. Then put the sample in a desiccator until cool and re-weighted to constant weight (W_2). The moisture content (MC) was then calculated on oven dry basis as a percentage according to the following equation:

$$\% \text{ Moisture content (\%MC)} = \frac{Mw \text{ (mass of water = } W_1 - W_2)}{W_1 \text{ (weight of initial sample)}} \times 100$$

III. Estimation of carbohydrates

1. Total carbohydrates

For determination of carbohydrates, anthrone sulphuric acid method was used according to Fales (1951), Schlegel (1956) and Badour (1959).

2. Water-soluble carbohydrates

To estimate the water-soluble carbohydrates, a known weight of the bagasse powder was extracted by distilled water for 2 hrs. in a boiling water bath. After cooling, the water-soluble carbohydrates were determined and calculated as mg/g dry weight.

3. Water insoluble carbohydrates

It was calculated as the difference between the amount of the total and water-soluble carbohydrates.

IV. Isolation of fungi

Glucophilic fungi of bagasse were isolated using the following techniques:

1. Dilution-plate method

Dilution-plate technique as described by Moubasher (1993), and Pitt & Hocking (2009) was employed to isolate fungi from sugarcane bagasse samples tested. Sugarcane bagasse was cut into very small pieces. Ten gm of the sample pieces were transferred into sterilized dilution glass contained 90 ml of sterile distilled water, then shaking well for 10 minutes using rotary shaker. Serial dilutions were prepared. Spread-plate technique was used in inoculation 10 plates for each sample with the desired prepared dilution. One ml of supernatant suspension of selected desired prepared dilution was transferred into each plate, spread, poured about 15-20 ml of sterilized used medium. The inoculated plates were rotated clockwise and anticlockwise to spread and thoroughly homogenate the suspension uniformly. Five replicates of Czapek's-glucose agar medium for each sample, then incubated at $28 \pm 2^\circ\text{C}$ for 7 days. The developing fungi were counted as colony forming units (CFUs), isolated and identified. Pure cultures of identified fungi were transferred to Czapek's agar slants and kept for further studies.

2. Direct plating method

Direct-plating technique as described by Pitt & Hocking (2009) was employed to isolate fungi from sugarcane bagasse samples tested. Sugarcane bagasse was cut in aseptic conditions into segments of 0.5 cm. Forty segments of each sample were transferred and fixed on surface plates of Czapek's-glucose agar and Czapek's-cellulose agar media. Five replicates for Czapek's-glucose agar medium of each sample tested were inoculated with 4 segments for each plate. The inoculated plates were incubated at $28 \pm 2^\circ\text{C}$ for 7 days. The developing fungi were counted as colony forming units (CFUs) per 20 segments for each sample, isolated and identified. Pure cultures of identified fungi were transferred into Czapek's-glucose agar slants and kept for further studies.

3. Media used in isolation of fungi

a) Czapek's-glucose agar medium (Cz)

Czapek's-glucose agar medium was used according to Smith & Dawson (1944), Martin (1950) & Al-Doory (1980) for isolation of glucophilic fungi, identification and maintenance of isolated fungi from sugarcane bagasse samples, and its composition for one liter is as

follow:- Glucose, 10.0g; NaNO₃, 3.0g; KH₂PO₄, 1.0g; MgSO₄.7H₂O, 0.5g; KCl, 0.5g; Agar, 20.0g; Distilled water, 1000 ml; Chloramphenicol, 0.5 mg/ml at PH, 6.2- 6.6.

V. Identification of isolated fungi

The morphological characteristics based on macro- and microscopic characteristics of hyphae and spores were used for identification of fungi to species level. The following scientific references were consulted for identification: Ames (1969), for *Chaetomium* species, Domsch et al. (2007), for fungi in general, Ellis (1971, 1976), for Dematiaceous Hyphomycetes, Leslie & Summerell (2006), for *Fusarium* species, Ismail et al., (2015), for *Fusarium* species, Moubasher (1993), for fungi in general, Pitt (1979, 1985), for *Penicillium* species Raper & Fennell (1965), for the genus *Aspergillus*.

Results and Discussion

The moisture content of sugarcane bagasse samples tested

Thirty sugarcane bagasse samples were collected from different sugarcane juice mills in Sohag Governorate. The moisture content of tested samples was fluctuated between 32.58 - 70.59%. The highest content was recorded in sample (No. 22), while the lowest was observed in sample (No. 10) as shown in table (1).

Table 1: The moisture content of tested sugarcane bagasse samples

SN	MC%	SN	MC%
1	49.47	16	60.75
2	49.44	17	47.47
3	48.71	18	41.98
4	53.2	19	47.17
5	65.58	20	62.58
6	35.47	21	67.14
7	33.98	22	70.59
8	47.21	23	67.99
9	36.33	24	64.82
10	32.58	25	49.43
11	45.12	26	53.20
12	51.80	27	59.29
13	41.56	28	59.95
14	52.05	29	48.62
15	43.98	30	44.95

SN: Sample number. MC%: Percentage of moisture content.

Moisture content

In the current study, the moisture content of the tested samples was fluctuated between 32.58-70.59%, but the majority of samples (56.66 %) fluctuated between 41.56- 53.2%. In this respect, these data are in full harmony with the results obtained by Manohar (1997)

who reported that wet mill bagasse had moisture 50%, fiber pith 47%, sugar 2.5% and mineral 0.5%. Also, Anwar (2010) stated that moisture content of mill sugarcane bagasse samples fluctuated between 46 - 52%, with average of 15 samples is 50.0%, while Chauhan et al., (2011) recorded that sugarcane bagasse contained 50% fiber, 48% moisture and 2% sugar which couldn't be extracted. As well as Yadav et al. (2015) in another study found that the moisture content of bagasse was 49%.

Total carbohydrates, soluble and insoluble sugars in tested sugarcane bagasse samples

Total carbohydrates were estimated, and the richest sample was (No.21), which contained 535.10 mg/g dry weight, whereas the lowest total carbohydrate sample was recorded in sample (No.3) with 508.131 mg/g dry weight.

Soluble sugars of different sugarcane bagasse samples were estimated using spectrophotometer at 620 nm. The soluble sugar ranged between 120 - 160 mg/g dry weight. The highest content of soluble sugars was observed in sample (No.22), whilst the lowest content was recorded in sample (No.9).

On the other hand, insoluble sugars were also determined as shown in table (2). The highest value was recorded in sample (No.6) with 389.922 mg/g dry weight, whereas the lowest value was shown in sample (No.1) with 369.444 mg/g dry weight.

Data in the current study were like that recorded by Vodo et al. (2020) who reported that total carbohydrates of raw bagasse was 636±0.1mg/g dry weight, while total carbohydrates of bagasse extract from hydrothermal liquefaction (HTL) treatment method was 510.3±0.1mg/g dry weight. Also, Zeinaly et al. (2017) stated that total carbohydrates of raw bagasse was 655.1 mg/g. On the other hand, Banerjee (2014) recorded those total carbohydrates of bagasse was 733 mg/g, i.e., nearly 73% of carbohydrate in bagasse was released.

Fungi recovered on glucose-Czapek's agar medium using dilution-plate method

Twenty-seven species and four species-varieties belonging to eight genera were recovered from 30 sugarcane bagasse samples on glucose-Czapek's agar medium at 28 ± 2°C using dilution-plate method (table, 3). The results indicated that sample No. 3 contained the highest total count, but the sample No. 4 recorded the lowest total count (8600 and 40 cfu/g dry weight). *Aspergillus* was represented by 18 species and 4 species-varieties with high frequencies of occurrence (96.7% of total samples and 74.15% of

total fungi). Of which *A. fumigatus* was the most abundant species, isolated in high frequency of occurrence (63.3% of the samples and 11.07% of total count of fungi), *A. aculeatus*, *A. flavus* and *A. japonicus* were isolated in moderate frequencies of occurrence (33.3, 26.7 and 26.7 % of the samples) and (6.05, 13.77 and 7.17 % of total count of fungi), respectively. *A. terreus*, *A. tubengensis*, *A. niger*, *A. parasiticus*, *A. heteromorphus* and *A. fotedius* var. *pallidus* were isolated in low frequencies of occurrence (23.3, 23.3, 20, 16.7, 16.7 and 16.7% of the samples) and (1.18, 1.61, 2.09, 9.67, 2.27 and 0.43% of total count of fungi), respectively.

Table 2: Total carbohydrates, soluble and insoluble sugars in tested sugarcane bagasse samples (mg/g dry weight).

Sample No	Total carbohydrates	Soluble sugars	Insoluble sugars
1	509.184	139.74	369.444
2	510.121	140.00	370.121
3	508.131	130.131	378
4	515.152	135.130	380.022
5	530.156	150.155	380.001
6	511.135	121.213	389.922
7	520.156	125.666	394.49
8	510.00	135.00	375
9	515.150	120.20	394.95
10	514.00	125.11	388.89
11	512.00	132.16	379.84
12	521.16	140.00	381.16
13	523.19	135.00	388.19
14	520.12	141.41	378.71
15	515.00	137.00	378
16	528.11	142.32	385.79
17	515.10	130.10	385
18	520.20	130.13	390
19	515.10	128.17	386.93
20	528.16	141.41	386.75
21	535.10	152.00	383.1
22	534.30	160.60	373.7
23	528.19	149.20	378.99
24	528.17	138.14	390.03
25	520.00	135.34	390.03
26	518.15	131.35	386.8
27	530.20	142.13	385.07
28	529.00	141.00	388
29	515.00	125.21	389.79
30	515.89	138.45	377.44

The remaining *Aspergillus* species were collected and identified in rare frequencies of occurrence (less than 4 samples) and these were *A. aneus*, *A. awamori*, *A. carbonarius*, *A. ficuum*, *A. flavus* var. *columinarius*, *A. fotedius*, *A. fotedius* var. *acidus*, *A. oryzae*, *A. phoenicis*, *A. pluvulentus*, *A. terreus* var. *aureus*.

Talaromyces was the second most frequent genus, recorded in moderate frequency of occurrence (36.7% of the samples and 19.97% of total fungi), represented by 3 species namely; *T. duclauxii*, *T. luteus* which

appeared in low frequencies of occurrence (13.3 & 16.7% of the samples, 11.67 & 6.88% of total fungi), respectively and *T. Purpurgenus* which collected in rare frequency of occurrence (6.7% of the samples and 1.32% of total fungi).

Acremonium occupied the third place followed by *Penicillium* in fourth place among fungal genera, each one represented by one species *Acremonium rutium* and *Penicillium lanosum* and these were collected in rare frequencies of occurrence, but *Penicillium* count was very low (10 and 10% of the samples, 4.13 and 0.89% of total fungi), respectively.

The remaining genera *Fusarium* (*F. oxysporum*), *Mucor* (*M. circinelliodes*), *Trichoderma* (*T. viride*) and *Verticillium* (*V. terrestre*) were isolated in rare frequencies of occurrence and low counts (3.3, 3.3, 6.7 and 3.3% of the samples), accounting (0.11, 0.03, 0.60 and 0.11% of total fungi), respectively.

Fungi recovered on glucose-Czapek's agar medium using direct- plate method

The results showed that the fungal counts were fluctuated between 13- 64 colonies (calculated per 20 segments in each sample tested). The richest sample was No.7 (64 colonies). The poorest sample was No. 30 (13 colonies). Also, infection percentage ranged between 0.65 % in sample No., 30 to 3.2% in sample No., 7 as shown in table (4).

Twenty-five species and four species varieties belonging to 8 genera were isolated from 30 sugarcane bagasse samples of which *Aspergillus* and *Talaromyces* the most common genera.

Aspergillus was the most common genus, occurring in (100% of the samples, 88.3% of total count of fungi). The best count was estimated in sample No. 14 (39 colonies), but the lowest count was estimated in sample No. 1 (30 colonies). It was represented by 18 species and 3 varieties of which, *A. flavus*, *A. niger*, *A. phoenicis* and *A. tubengensis* were the most dominant species and collected in moderate frequencies of occurrence (46.7, 36.7, 26.7 and 26.7% of the samples and 16.8, 12.3, 4.8 and 9.8% of total fungi), respectively.

A. aculateatus, *A. awamori*, *A. ellepticus*, *A. ficuum*, *A. fumigatus*, *A. japonicus*, *A. oryzae*, *A. parasiticus*, *A. pluvulentus*, *A. terreus* were collected in low frequencies of occurrence between 4- 7 samples (20, 20, 13.3, 20, 23.3, 20, 13.3, 13.3, 13.3 and 13.3% of the samples and 4.3, 4.5, 3.6, 2.9, 2.2, 3.5, 4, 3.5, 3.7 and 1.7% of total fungi), respectively.

A. avenaceus, *A. carbonarius*, *A. flavus* var. *columinarius*, *A. fotedius*, *A. fotedius* var. *acidus*, *A. fotedius* var. *pallidus* and *A. terreus* var. *aureus* were isolated and identified in rare frequencies of occurrence (less than 4 samples out of 30 tested).

Talaromyces was the second most dominant genus and was represented in (40% of the samples and 3.26% of total fungi). Two species were identified namely; *T. duclauxii* and *T. purpurgenus* which collected in moderate and low frequencies of occurrence, respectively (26.7 and 13.3% of the samples and 2.8 and 0.5% of total fungi), respectively. Six genera were collected and identified in rare frequencies of

occurrence namely; *Acremonium*, *Curvularia*, *Fusarium*, *Mucor*, *Penicillium* and *Verticillium* each one was represented only by one species; *Acremonium rutilum*, *Curvularia specifera*, *Fusarium oxysporum*, *Mucor circinelliodes*, *Penicillium nigricans* and *Verticillium terrestre* (10, 3.3, 3.3, 10, 3.3 and 6.7% of the samples and 1.8, 0.1, 0.8, 1, 0.12 and 0.6% of total fungi), respectively.

Table 3: Total counts (calculated per g dry weight sugarcane bagasse) of fungal genera and species recovered from 30 bagasse samples on glucose–Czapek’s agar at 28±2°C, using dilution–plate method.

Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Fungal taxa															
Total count	8500	5200	8600	40	260	880	1560	3340	1940	780	1840	580	4780	1620	560
<i>Acremonium rutilum</i>	800	2000	0	0	0	0	0	0	0	80	0	0	0	0	0
<i>Aspergillus</i>	6300	600	400	0	220	820	1560	3340	1920	780	1840	580	4780	1620	560
<i>A. aculeatus</i>	0	0	0	0	0	0	0	232	40	0	32	0	244	184	0
<i>A. aeneus</i>	0	0	0	0	0	0	0	0	16	0	0	0	0	0	0
<i>A. awamori</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. carbonarius</i>	0	0	0	0	0	0	0	0	0	0	32	0	0	0	0
<i>A. ellepticus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. ficuum</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. flavus</i>	5600	0	400	0	0	720	1320	0	0	0	240	0	740	0	0
<i>A. flavus var. columnaris</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. fotedius</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. fotedius var. acidus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. fotedius var. pallidus</i>	0	0	0	0	0	0	0	0	8	0	0	24	0	0	0
<i>A. fumigatus</i>	200	600	0	0	0	80	240	0	100	40	1160	40	1860	0	120
<i>A. heteromorphus</i>	0	0	0	0	0	0	0	0	0	0	0	120	0	0	0
<i>A. japonicus</i>	0	0	0	0	0	0	0	1160	60	0	0	100	1020	700	0
<i>A. niger</i>	0	0	0	0	100	0	0	0	100	0	0	140	0	0	440
<i>A. oryzae</i>	0	0	0	0	0	0	0	1020	0	0	0	0	0	0	0
<i>A. parasiticus</i>	300	0	0	0	0	0	0	0	1160	0	0	0	0	0	0
<i>A. phoenicis</i>	0	0	0	0	0	0	0	0	0	0	260	0	0	0	0
<i>A. pluverulentus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. terreus</i>	200	0	0	0	0	0	0	0	0	120	20	0	20	0	0
<i>A. terreus var. Aureus</i>	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0
<i>A. tubengensis</i>	0	0	0	0	120	0	0	0	180	620	0	60	20	0	0
<i>Fusarium oxysporum</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mucor circinelliodes</i>	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0
<i>Penicillium lanosum</i>	0	400	200	0	20	0	0	0	0	0	0	0	0	0	0
<i>Talaromyces</i>	1000	2200	8000	20	0	60	0	0	0	0	0	0	0	0	0
<i>T. duclauxii</i>	800	0	7000	0	0	0	0	0	0	0	0	0	0	0	0
<i>T. luteus</i>	0	2200	1000	20	0	60	0	0	0	0	0	0	0	0	0
<i>T. purpurogenus</i>	200	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichoderma viride</i>	400	0	0	0	20	0	0	0	0	0	0	0	0	0	0
<i>Verticillium terrestre</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3: Continued

Samples	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Fungal taxa															
Total count	80	1880	1440	2460	2660	4980	1260	860	4160	4620	880	1360	620	840	200
<i>Acremonium rutilum</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aspergillus</i>	80	1880	1440	2460	840	4980	1160	860	4160	4620	880	1360	620	840	200
<i>A. aculeatus</i>	0	0	0	40	40	0	0	0	0	40	0	580	0	120	0
<i>A. aeneus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. awamori</i>	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. carbonarius</i>	0	0	0	0	0	0	0	0	520	120	0	0	0	0	0
<i>A. ellipticus</i>	0	1280	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. ficuum</i>	0	0	20	60	0	0	0	0	0	0	0	0	0	0	140
<i>A. flavus</i>	0	0	0	0	220	0	360	0	0	0	0	0	0	0	0
<i>A. flavus</i> var. <i>columnaris</i>	0	0	0	2240	0	3200	0	0	0	0	0	0	0	0	0
<i>A. fotedius</i>	0	0	20	0	0	0	280	0	0	0	0	0	0	0	0
<i>A. fotedius</i> var. <i>acidus</i>	0	0	0	0	20	0	0	0	0	0	100	0	0	0	0
<i>A. fotedius</i> var. <i>pallidus</i>	0	0	0	0	20	0	0	80	0	0	0	40	0	0	0
<i>A. fumigatus</i>	80	0	260	80	260	0	280	0	0	580	780	480	480	0	0
<i>A. heteromorphus</i>	0	600	0	0	0	0	0	360	460	40	0	0	0	0	0
<i>A. japonicus</i>	0	0	0	0	0	1780	0	0	0	40	0	0	140	0	0
<i>A. niger</i>	0	0	0	0	0	0	0	420	0	0	0	260	0	0	0
<i>A. oryzae</i>	0	0	1120	0	0	0	0	0	0	2340	0	0	0	0	0
<i>A. parasiticus</i>	0	0	0	0	0	0	0	0	3180	1460	0	0	0	640	0
<i>A. phoenicis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	60	40
<i>A. pluverulentus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20
<i>A. terreus</i>	0	0	0	0	200	0	240	0	0	0	0	0	0	20	0
<i>A. terreus</i> var. <i>Aureus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. tubengensis</i>	0	0	0	40	80	0	0	0	0	0	0	0	0	0	0
<i>Fusarium oxysporum</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	80
<i>Mucor circinelliodes</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Penicillium lanosum</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Talaromyces</i>	0	0	0	0	1820	0	20	0	0	0	0	0	0	144	0
<i>T. duclauxii</i>	0	0	0	0	300	0	20	0	0	0	0	0	0	0	0
<i>T. luteus</i>	0	0	0	0	1520	0	0	0	0	0	0	0	0	0	0
<i>T. purpurogenus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	720	0
<i>Trichoderma viride</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Verticillium terrestre</i>	0	0	0	0	0	0	0	0	0	0	0	80	0	0	0

In comparison of mycobiota of bagasse samples recovered by dilution- and direct- plate methods on glucose-Czapek's agar medium at 28 ±2°C, it was found that the total fungal populations in case of dilution-plate method was so high than those of direct-plate method (69720 & 828 colonies, respectively), these agree with El-Kady & Youssef (1993) who used these two methods for isolation of fungi from soybean seeds and reported that the total count of fungal population at 28°C was so high in case of dilution- plate method (25034 cfu per g dry seeds) than another method (6061 colonies per 25 seeds). *Aspergillus* was also appeared in high population and was parallel to those of total fungal counts (51700 colonies/gm, 74.15 % of total count, in 29 samples with 96.7% of the samples in dilution method and 731 colonies/ 20 segments, 88.3% of total count, in 30 samples with 100% of the samples tested using direct-

plate method.

A. flavus was isolated with high total counts in both dilution and plating methods (9600 & 139 colonies, respectively), but was appeared in moderate frequencies of occurrence in both two methods (26.7 & 47.6% of samples).

Some fungal species were appeared only on the dilution-plate method and completely missed on direct-plate method, and these were *A. aeneus*, *A. heteromorphus*, *Talaromyces luteus* and *Trichoderma viride* as shown in table (5). Other species were completely absent when dilution-plate method was used and appeared only on direct-plate method such as *A. avenaceus*, *P. nigricans* and *Curvularia specifera*.

On the other hand, *A. fumigatus* was appeared in high frequency of occurrence (63%) in case of dilution-plate method whereas, in low frequency of occurrence

in case of direct-plate method (23.3 %). *A. aculeatus* and *A. japonicus* were appeared in moderate frequencies in case of dilution-plate method, whereas, in low frequencies in case of direct plate method.

Aspergillus niger, *A. tubengensis* and *Talaromyces duclauxii* were appeared in moderate frequencies in direct-plate method, while in low frequencies in

dilution-plate method, as well as *A. awamori*, *A. ficuum*, *A. oryzae*, *A. pluverulentus* and *T. purpurogenus* were appeared in low frequencies in direct-plate method but, in rare frequencies in case of dilution-plate method.

Table 4: Total counts (calculated per 20 sugarcane bagasse segments in each sample) of common fungal genera and species recovered from 30 bagasse samples on glucose–Czapek’s agar medium at 28±2°C, using direct-plate method.

Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Fungal taxa															
Total count	36	47	21	32	23	36	64	30	17	27	23	17	27	39	24
<i>Acremonium rutilum</i>	0	13	0	1	0	0	1	0	0	0	0	0	0	0	0
<i>Aspergillus</i>	30	34	21	30	22	36	27	22	17	26	23	17	27	39	23
<i>A. aculeatus</i>	0	0	0	0	0	0	4	0	0	0	0	2	4	0	0
<i>A. avenaceus</i>	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0
<i>A. awamori</i>	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. carbonarius</i>	0	0	0	0	0	0	0	5	10	0	0	0	0	0	0
<i>A. ellipticus</i>	0	0	0	0	0	7	0	0	0	0	0	0	0	0	7
<i>A. ficuum</i>	0	0	0	0	0	0	0	0	0	0	0	4	0	0	4
<i>A. flavus</i>	10	11	0	0	0	19	15	0	0	8	0	0	0	19	0
<i>A. flavus</i> var. <i>columnaris</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. fotedius</i>	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0
<i>A. fotedius</i> var. <i>acidus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. fotedius</i> var. <i>pallidus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. fumigates</i>	0	0	0	0	0	0	1	2	0	0	3	1	7	0	0
<i>A. japonicus</i>	0	0	0	0	0	0	0	5	0	4	0	2	12	0	0
<i>A. niger</i>	0	0	0	20	19	0	7	0	0	0	0	0	0	0	3
<i>A. oryzae</i>	0	0	19	3	0	0	0	0	0	0	0	0	0	0	9
<i>A. parasiticus</i>	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0
<i>A. phoenicis</i>	0	0	0	0	0	10	0	5	0	0	2	4	0	0	0
<i>A. pluverulentus</i>	0	0	0	0	0	0	0	0	0	0	2	0	4	20	0
<i>A. terreus</i>	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. terreus</i> var. <i>aureus</i>	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. tubengensis</i>	8	18	0	0	0	0	0	5	7	14	16	0	0	0	0
<i>Curvularia specifiers</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fusarium oxysporum</i>	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0
<i>Mucor circinelliodes</i>	6	0	0	0	0	0	0	1	0	0	0	0	0	0	0
<i>Penicillium nigricans</i>	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
<i>Talaromyces</i>	0	0	0	1	1	0	0	1	0	1	0	0	0	0	1
<i>T. duclauxii</i>	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
<i>T. purpurogenus</i>	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0
<i>Verticillium terrestre</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Infection Percentage %	1.8	2.35	1.05	1.60	1.15	1.80	3.20	1.50	0.85	1.35	1.15	0.85	1.35	1.95	1.20

Table 4: Continued

Samples	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Fungal taxa															
Total count	16	20	27	29	14	37	21	35	21	21	30	33	26	22	13
<i>Acremonium rutilum</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aspergillus</i>	16	20	24	29	14	37	21	35	21	16	23	31	24	18	8
<i>A. aculeatus</i>	0	0	0	0	3	18	0	0	0	5	0	0	0	0	0
<i>A. avenaceus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. awamori</i>	4	4	11	0	3	0	0	0	0	0	3	0	0	0	0
<i>A. carbonarius</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. ellepticus</i>	0	8	0	0	0	0	8	0	0	0	0	0	0	0	0
<i>A. ficuum</i>	4	0	0	4	4	0	0	0	0	0	0	0	4	0	0
<i>A. flavus</i>	3	0	10	7	0	19	5	11	1	0	0	0	0	0	1
<i>A. flavus</i> var. <i>columnaris</i>	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0
<i>A. fotedius</i>	0	0	0	6	0	0	8	16	0	0	0	0	0	0	0
<i>A. fotedius</i> var. <i>acidus</i>	0	0	0	0	3	0	0	0	0	0	3	0	0	0	0
<i>A. fotedius</i> var. <i>pallidus</i>	0	8	0	0	1	0	0	0	0	0	6	0	0	0	0
<i>A. fumigatus</i>	0	0	0	2	0	0	0	0	0	0	0	0	0	2	0
<i>A. japonicus</i>	4	0	0	0	0	0	0	0	0	0	2	0	0	0	0
<i>A. niger</i>	0	0	0	1	0	0	0	8	20	5	0	10	7	2	0
<i>A. oryzae</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
<i>A. parasiticus</i>	0	0	0	0	0	0	0	0	0	0	9	14	0	3	0
<i>A. phoenicis</i>	0	0	0	4	0	0	0	0	0	0	0	0	4	4	7
<i>A. pluverulentus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0
<i>A. terreus</i>	1	0	3	5	0	0	0	0	0	0	0	0	0	0	0
<i>A. terreus</i> var. <i>aureus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. tubengensis</i>	0	0	0	0	0	0	0	0	0	6	0	7	0	0	0
<i>Curvularia specifera</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
<i>Fusarium oxysporum</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mucor circinelliodes</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>Penicillium nigricans</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Talaromyces</i>	0	0	3	0	0	0	0	0	0	5	7	1	2	3	1
<i>T. duclauxii</i>	0	0	3	0	0	0	0	0	0	5	7	1	2	3	0
<i>T. purpurogenus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>Verticillium terrestre</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	0	4
Infection Percentage %	0.80	1.00	1.35	1.45	0.70	1.85	1.05	1.75	1.05	1.05	1.50	1.65	1.3	1.10	0.65

A. terreus and *A. parasiticus* were appeared in low frequencies in both dilution and direct-plate methods. As well as some species were appeared in rare frequencies of occurrence in both two methods such as *Acremonium rutilum*, *Aspergillus carbonarius*, *A. flavus* var. *columnaris*, *A. fotedius*, *A. fotedius* var. *acidus*, *A. terreus* var. *aureus*, *Fusarium oxysporum*, *Mucor circinelliodes* and *Verticillium terrestres* shown in table (5).

Thirty Species and four species varieties belonging to nine genera were recovered from 30 sugarcane bagasse samples on glucose-Czapek's agar at 28°C by using two methods of isolation. The results are in harmony with that obtained by Abdel-Hafez *et al.* (1995) who isolated 46 species and 2 varieties belonging to 20 genera from 50 sugarcane juice samples on glucose-, sucrose- and cellulose-Czapek's agar at 28°C. Also, Moretti *et al.* (2012) reported that the lignocellulosic biomass in this residue can limit the number of microorganisms, restricting the presence of species which are not able to produce hydrolytic enzymes.

In the current results, the most frequently encountered genera isolated from sugarcane bagasse samples using the two methods of isolation were *Aspergillus*, *Talaromyces*, *Acremonium* and *Penicillium*. But *Aspergillus* is the most common genus that represented by 18 species and 4 species varieties. From which, *A. fumigatus* was isolated in high occurrence from sugarcane bagasse samples using dilution plate method, but in low occurrence in direct plating method of isolation. Also, *A. flavus* was isolated in moderate frequencies of occurrence in both two methods of isolation, as well as *A. niger*, *A. phoenicis* and *A. Tubengensis* were isolated in moderate frequencies of occurrence in direct plating method. These *Aspergillus* species were previously recovered from sugarcane leaves, stem, bagasse and juice by Higgy *et al.* (1977), Sandhu *et al.* (1980), Olufolaji (1986), Sivanesan & Waller (1986), Muhsin & Abdel-Kader (1995) and Abdel-Hafez *et al.* (1995). *Aspergillus* strains with environmental and economic importance have previously been described (Baffi *et al.*, 2012; Souza *et*

al., 2012). Strains from this genus are also known as good producers of extracellular enzymes, giving these microorganisms great potential for growth in wastes (Jaafaru *et al.*, 2007 and Gottschalk *et al.*, 2013).

The abundance of *A. fumigatus* on bagasse is in conformity with the earlier reports by Sandhu *et al.* (1977 & 1980). Also (Souza *et al.*, 2012) found that *A.*

fumigatusis an ubiquitous fungus commonly found in agricultural byproducts, and these agree with the current results. This species is one of a wide range of fibrolytic enzyme-producing organisms, and some strains have been described as having great potential for cellulase and hemicellulase production (Moretti *et al.*, 2012; Youssef *et al.*, 2016).

Table 5: Comparison between total counts (calculated per g dry weight sugarcane bagasse in every sample); percentage counts (per total fungi); numbers of cases of isolation (out of 30) and occurrence remarks of fungal genera and species recovered from 30 sugarcane bagasse samples on glucose–Czapek’s agar at 28±2°C, using dilution- and direct-plate methods.

Fungal taxa	Dilution-plate method					Direct-plate method				
	TC	TC%	F%	NCI	OR	TC	TC%	F%	NCI	OR
Total count	69720					828				
<i>Acremonium rutilum</i> W. Gams	2880	4.13	10.0	3	R	15	1.8	10.0	3	R
<i>Aspergillus</i>	51700	74.15	96.7	29	H	731	88.3	100	30	H
<i>A. aculeatus</i> Iizuka	4220	6.05	33.3	10	M	36	4.3	20.0	6	L
<i>A. aeneus</i> Sappa	80	0.11	3.3	1	R	-	-	-	-	-
<i>A. avenaceus</i> Smith	-	-	-	-	-	7	0.8	3.3	1	R
<i>A. awamori</i> Nakazawa	20	0.03	3.3	1	R	37	4.5	20.0	6	L
<i>A. carbonarius</i> (Bainier) Thom	800	1.15	10.0	3	R	15	1.8	6.7	2	R
<i>A. ellepticus</i> , sp.nov.	1280	1.84	3.3	1	R	30	3.6	13.3	4	L
<i>A. ficuum</i> (Reich.) Hennings	220	0.32	10.0	3	R	24	2.9	20.0	6	L
<i>A. flavus</i> Link	9600	13.77	26.7	8	M	139	16.8	46.7	14	M
<i>A. flavus</i> var. <i>columnaris</i> Raper & Fennell	5440	7.80	6.7	2	R	9	1.1	3.3	1	R
<i>A. fotedius</i> (Naka.) Thom and Raper	300	0.43	6.7	2	R	34	4.1	13.3	4	R
<i>A. fotedius</i> var. <i>acidus</i> N., S., & W	120	0.17	6.7	2	R	6	0.7	6.7	2	R
<i>A. fotedius</i> var. <i>pallidus</i> N., S., & W	300	0.43	16.7	5	L	15	1.8	10.0	3	R
<i>A. fumigatus</i> Fresenius	7720	11.07	63.3	19	H	18	2.2	23.3	7	L
<i>A. heteromorphus</i> Batista and Maia	1580	2.27	16.7	5	L	-	-	-	-	-
<i>A. japonicus</i> Saito	5000	7.17	26.7	8	M	29	3.5	20.0	6	L
<i>A. niger</i> V. Tieghem	1460	2.09	20.0	6	L	102	12.3	36.7	11	M
<i>A. oryzae</i> (Ahlb.) Cohn	4480	6.43	10.0	3	R	33	4	13.3	4	L
<i>A. parasiticus</i> Speare	6740	9.67	16.7	5	L	29	3.5	13.3	4	L
<i>A. phoenicis</i> (Corda) Thom	360	0.52	10.0	3	R	40	4.8	26.7	8	M
<i>A. pluverulentus</i> (Mc-Alp.) Thom	20	0.03	3.3	1	R	31	3.7	13.3	4	L
<i>A. terreus</i> Thom	820	1.18	23.3	7	L	14	1.7	13.3	4	L
<i>A. terreus</i> var. <i>aureus</i> Thom&Raper	20	0.03	3.3	1	R	2	0.2	3.3	1	R
<i>A. tubengensis</i> (Schober) Moss.	1120	1.61	23.3	7	L	81	9.8	26.7	8	M
<i>Curvularia specifiara</i> (Bainier) Boedijin	-	-	-	-	-	1	0.1	3.3	1	R
<i>Fusarium oxysporum</i> Schlechtenda	80	0.11	3.3	1	R	7	0.8	3.3	1	R
<i>Mucor circinelliodes</i> (Van Tieghem)	20	0.03	3.3	1	R	8	1	10.0	3	R
<i>Penicillium</i> Thom&Raper	620	0.89	10.0	3	R	1	0.12	3.3	1	R
<i>P. lanosum</i> Westling	620	0.89	10.0	3	R	-	-	-	-	-
<i>P. nigricans</i> (Bainier) Thom	-	-	-	-	-	1	0.1	3.3	1	R
<i>Talaromyces</i>	13920	19.97	36.7	11	M	27	3.26	40.0	12	M
<i>T. duclauxii</i> Delacroxi	8200	11.76	13.3	4	L	23	2.8	26.7	8	M
<i>T. luteus</i> (Zukal) Benjamin	4800	6.88	16.7	5	L	-	-	-	-	-
<i>T. purpurogenus</i> Stool	920	1.32	6.7	2	R	4	0.5	13.3	4	L
<i>Trichoderma viride</i> Pers. ex S.F. Gray	420	0.60	6.7	2	R	-	-	-	-	-
<i>Verticillium terrestre</i> (Link) Lindau	80	0.11	3.3	1	R	5	0.6	6.7	2	R
No. of genera, species and species-varieties	8 genera, 27 species and 4 varieties					8 genera, 25 species and 4 varieties				

TC = Total count.

TC % = Percentage of total count. NCI = Numbers of cases of isolation.

OR = Occurrence Remarks: H = High occurrence; more than 14 samples, M = Moderate occurrence; between 8 – 14 samples,

L = Low occurrence; between 4 – 7 samples, R = Rare occurrence; < 4 samples.

Furthermore, species of *Talaromyces*, *Penicillium*, *Fusarium*, *Mucor*, *Curvularia*, *Chaetomium* and *Trichoderma* were identified during this investigation. These results are in full agreement with Boonyuen *et al.* (2014), who recorded *A. fumigatus*, *A. niger*, *A. flavus*, *A. tubengencies* and species of *Talaromyces*, *Penicillium*, *Fusarium*, *Mucor*, *Curvularia*, *Chaetomium* and *Trichoderma* from sugarcane filter cake and bagasse isolated from sugar refineries in Thailand.

Some of the observed species are considered to be rare in this study. such as, *Penicillium* species. These results are in agreement with that reported by Domsch *et al.* (2007) who stated that although *Penicillium* spores are likely to be found dispersed in the air in all environments, these species are more common in regions where low temperatures prevail. Some fungi reported here, including *A. niger*, *A. terreus*, *A. fumigatus*, were similar to those reported by Cortés-Espinosa *et al.* (2006), who studied the selection, identification and application of fungi isolated from sugarcane bagasse (SBG) in Mexico.

Trichoderma spp. have commonly been found to be associated with sugarcane bagasse El-Amin & Saadabi (2007), Deshmukh *et al.* (2013), Romao-Dumaresq *et al.* (2016) and Souza *et al.* (2016).

Also, *Aspergillus*, *Acremonium*, *Alternaria*, *Curvularia*, *Fusarium*, *Penicillium*, *Chaetomium* and *Mucor* were isolated from leaf, stalk, root and rhizosphere of sugarcane plant by different researchers Abdel-Rahim *et al.* (1983), El-Amin & Saadabi (2007), Stuart *et al.* (2010), Deshmukh *et al.* (2013), Romão-Dumaresq *et al.* (2016) and Souza *et al.* (2016).

Some *Fusarium* species are known to be associated with sugarcane, establishing either symbiotic or pathogenic relationships; *F. moniliforme* is the causal agent of both Pokkah-boeng and stem rot, and *F. sacchari* is the causal agent of sugarcane wilt. On the other hand, *F. oxysporum* strains have been reported as sugarcane endophytes (Stuart *et al.*, 2010 and Romao-Dumaresq *et al.*, 2016).

References

- Abdel-Hafez, S. I. I., El-Said, A. H., Gherbawy, Y. A. M. H. (1995). Mycoflora of leaf surface, stem, bagasse and juice of adult sugarcane plant and cellulolytic ability in Egypt. *Bulletin of Faculty of Science, Assiut University*, 24: 113-130.
- Abdel-Rahim, A. M., Baghadadi, A. M., Abdalla, M. H. (1983). Studies on fungus flora in the rhizosphere of sugarcane plants. *Mycopathologia*, 81: 183–186.
- Al-Doory, Y. (1980). *Laboratory medical mycology*. Leaand Febiger, Philadelphia Kimpton Publishers, London, pp. 410.
- Ames, L. A. (1969). *A monograph of the chaetomiaceae*. Weldon and Wesley, L. T. D., New York, pp. 65.
- Anwar, S. I. (2010). Determination of moisture content of bagasse of jaggery unit using microwave. *Journal of Engineering Science and Technology*, 5(4): 472 – 478.
- Badour, S. S. A. (1959). *Analytisch-Chemische untersuchung des kalimangles biechlorellsinvergleich mit anderon Mangel Zumstanden*. Ph.D. Dissertation Goettingen.
- Baffi, M. A., Romo-Sanchez, S., Ubeda-Iranzo, J. (2012). Fungi isolated from olive ecosystems and screening of their potential biotechnological use. *New Biotechnology Journal*, 29: 451-456.
- Banerjee, P. N. (2014). Comparative Analysis of Sugarcane Baggase Using Different Methods. *International Journal of Scientific & Engineering Research*, 5(9): 134- 137.
- Boonyuen, N., Manoch, L., Chamswarn, C., J. Luangsaard J., Piasai, O., Sri-indrasutdhi, V., Ueapattanakit, J., Chuaseeharonnachai, C. (2014). Fungal Occurrence on Sugarcane Filter Cake and Bagasse Isolated From Sugar Refineries in Thailand. *Thai Journal of Agricultural Science*, 47 (2): 77-86.
- Chauhan, M. K., Varun, C. S., Suneel, K. S. (2011). Life cycle assessment of sugar industry: A review. *Renewable and Sustainable Energy Reviews*, 15: 3445-3453.
- Cortés-Espinosa, D. V., Fernández-Perrino, F. J., Arana-Cuenca, A., Esparza-García, F., Loera, O., Rodríguez-Vázquez, R. (2006). Selection and identification of fungi isolated from sugarcane bagasse and their application for phenanthrene removal from soil. *Journal of Environmental Science and Health-Part A Toxic/Hazardous Substances and Environmental Engineering*, 41: 475-486.
- Deshmukh, R. B., Dange, S. S., Jadhav, P. V., Deokule, S. S., Patil, N. A. (2013). Studies on the mycoflora in the rhizosphere of sugarcane (*Saccharum officinarum* L.) *International Journal of Bioassays*, 2: 674–676.
- Domsch, K. H., Gam, W., Anderson, T. H. (2007). *Compendium of Soil Fungi*. 2nd ed., IHW Verlag, Eching bei München, pp. 672.

- El-Amin, N. A., Saadabi, S. (2007). Contribution to the knowledge of soil fungi in Sudan rhizosphere mycoflora of sugarcane at Kenana sugar state. *International Journal of Botany*, 3: 97–102.
- El-Kady, I. A., Youssef, M. S. (1986). Survey of mycoflora and mycotoxins in Egyptian soybean seeds. *Journal of Basic Microbiology*, 33: 371-378.
- Ellis, M. B. (1976). *More Dematiaceous Hypomycetes*. Commonwealth Mycological Institute, Kew, Surrey, England, pp.481.
- Fales, F. W. (1951). The assimilation and degradation of carbohydrates by yeast cells. *Journal of Biological Chemistry*, 193(1): 113-124.
- Gassara, F., Brar, S. K., Tyagi, R. D., Verma, M., Surampalli, R. Y. (2010). Screening of agro-industrial wastes to produce ligninolytic enzymes by *Phanerochaete chrysosporium*. *Biochemical Engineering Journal*, 49: 388-394.
- Gottschalk, L. M. F., Paredes, R. S., Teixeira, R. S. S. (2013). Efficient production of lignocellulolytic enzymes xylanase, -xylosidase, ferulic acid esterase and -glucosidase by the mutant strain *Aspergillus awamori* 2B.361 U2/1. *Brazilian Journal of Microbiology*, 44: 569-576.
- Hamada, Y. M. (2011). Water Resources Reallocation in Upper and Middle Egypt. EWRA European Water, EW Publications, 33: 33-44.
- Higgy, A. H., Abdel-Razik, A. A., Rushdi, H. M. (1977). Occurrence of pokkah boeng disease of sugarcane in ARE. 155CT.XVI-Congress Brazil, Plant Pathology Sec, 1: 473-481.
- Ismail, M. A., Abdel-Hafez, S. I. I., Hussein, N. A., Abdel-Hameed, N. A. (2015). Contributions to the genus *Fusarium* in Egypt with dichotomous keys for identification of species. Tomasz M. Karpiński, Poland, pp. 175.
- Jaafaru, M. I., Fagade, O. E. (2007). Cellulase Production and Enzymatic Hydrolysis of Some Selected Local Lignocellulosic Substrates by a Strain of *Aspergillus niger*. *Research Journal of Biological Sciences*, 2: 13-16.
- Leslie, J. F., Summerell, B. A. (2006). *The Fusarium Laboratory Manual*. Blackwell Publishing, pp. 388.
- Manohar, R. P. J. (1997). *Industrial utilization of sugar and its co-products*. New Delhi, India: ISPCK Publishers and distributors.
- Martin, J. P. (1950). Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. *J. Soil Science*, 69: 215- 233.
- Milala, M. A., Shugaba, A., Gidado, A., Ene, A. C., Wafar, J. A. (2005). Studies on the use of agricultural wastes for cellulose enzyme production by *Aspergillus niger*. *Research Journal of Agriculture and Biological Sciences*, 1(4): 325-328.
- Moretti, M. M. S., Martins, D. A. B., Da Silva, R. (2012). Selection of thermophilic and thermotolerant fungi for the production of cellulases and xylanases under solid-state fermentation. *Brazilian Journal of Microbiology*, 43: 1062-1071.
- Moubasher, A. H. (1993). *Soil fungi in Qatar and other Arab Countries*. University of Qatar, Centre for Scientific and Applied Research, pp. 566.
- Muhsin, T., Abdul-Kader, M. (1995). Ecology of fungi associated with *Phragmites australis* in Iraq. *Abhath Al-Yarmouk*, 4: 31-50.
- Olufolaji, D. B. (1986). *Curvularia* leaf spot of sugarcane. A new disease. *Sugarcane* 2: 1-2.
- Pandey, A., Carlos, R. S., Poonam, N., Vanete T. S. (2000). Biotechnological potential of agro-industrial residues. I: sugarcane Bagasse, *Bioresource Technology*, 74: 69-80.
- Pitt, J. I. (1979). The Genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Academic Press, London (pp. 634).
- Pitt, J. I., Hocking A. D. (2009). *Fungi and Food Spoilage*, 3rd ed. Springer- Verlag New York Inc. pp. 540.
- Rajoka, M. I. (2005). Regulation of synthesis of endo-xylanase and β -xylosidase in *Cellulomonas flavigena*: a Kinetic study. *World Journal of Microbiology and Biotechnology*, 21(4): 463-469.
- Raper, K. B., Fennell, D. I. (1965). *The genus Aspergillus*. Baltimore, Williams and Wilkins, pp. 686
- Romao-Dumaresq, A. S., Dourado, M. N., Favaro, L. C., Mendes, R., Ferreira, A., Araujo, W. L. (2016). Diversity of cultivated fungi associated with conventional and transgenic sugarcane and the interaction between endophytic *Trichoderma virens* and the host plant. *PLoS One*, 11:e0158974.

- Sandhu, D. K., Sidhu, M. S. (1980). Fungal succession on decomposing sugarcane bagasse. *Transactions of the British Mycological Society*, 75(2): 281-286.
- Sandhu, D. K., Singh, S., Waraich, M. K. (1980). Thermophilous fungi of decomposing sugarcane bagasse. *Canadian Journal of Botany*, 58: 2015-2016.
- Schlegel, H. G. (1956). Die Verwertung Organischer Sauren durch Chlorella ir Lichet. *Prlanta*, 47: 510.
- Sivanesan, A., Waller, J. (1986). Sugarcane diseases. *Phytopathological paper No.29*: 88.
- Smith, N. R., Dawson, V. T. (1944). The bacteriostatic action of rosebengal in medium used for the plate count of soil fungi. *Soil Science*, 58: 467-471.
- Souza, D. T., Bispo, A. S., Bon, E. P. (2012). Production of thermophilic endo-beta-1,4-xylanases by *Aspergillus fumigatus* FBSPE-05 using agro-industrial by-products. *Applied Biochemistry and Biotechnology*, 166: 1575-1515.
- Souza, R. S. C., Okura, V. K., Armanhi, J. S. L., Jorrin, B., Silva, M. J., Gonzalez-Guerrero, M., Araujo, L. M. (2016). Unlocking the bacterial and fungal communities assemblages of sugarcane microbiome. *Scientific Reports*, 6:28774.
- Stuart, R. M., Romao, A. S., Pizzirani-Kleiner, A. A., Azevedo, J. L., Araujo, W. L. (2010). Culturable endophytic filamentous fungi from leaves of transgenic imidazolinone-tolerant sugarcane and its non-transgenic isolines. *Archives of Microbiology*, 192:307-313.
- Van Reeuwijk, L. (2002). Procedures for soil analysis. International Soil Reference and Information Centre. Technical paper 9-120.
- Vodo, S., Taarji, N., Bouhoute, M., Felipe, L., Neves, M. A., Kobayashi, I., Uemura, K., Nakajima, M. (2020). Potential of bagasse obtained using hydrothermal liquefaction pretreatment as a natural emulsifier, *International Journal of Food Science and Technology*, 55: 1485-1496.
- Yadav, S., Gupta, G., Ravi Bhatnagar, R. (2015). A Review on composition and properties of bagasse fibers, *International Journal of Scientific & Engineering Research*, 6(5): 143-147.
- Youssef, M. S., El-Maghraby, O. M., Hassan, A. A., Rashwan, M. A. A. (2016). Mesophilic Mycobiota of composted Sorghum Wastes in Egypt, *Journal of Environmental Studies*, 15: 67-76.
- Zeinaly, F., Saraeian, A., Gabov, K., Pedro Fardim, P. (2018). Determination of carbohydrates in sugarcane bagasse pulp in different TCE bleaching sequences, *Cellulose Chemistry and Technology*, 51(1-2), 45-53.