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# Glucophilic fungi of sugarcane bagasse in Egypt

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**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

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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:

$$\% \text{ Moisture content (\%MC)} = \frac{\text{Mw (mass of water = W1- W2)}}{\text{W1 (weight of initial sample)}} \times (100)$$

### 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 ± 2°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 ± 2°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; $NaNO_3$, 3.0g; $KH_2PO_4$,1.0g; $MgSO_4.7H_2O$, 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. columinaris, *A. fotedius*, *A. fotedius* var. acidus, *A. oryzae*, *A. phoenicies*, *A. pluverulentus*, 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 rutlium 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. pluverulentus*, *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 (26.7and 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 specifiera*, *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** / **Fungal taxa** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Total count** | 8500 | 5200 | 8600 | 40 | 260 | 880 | 1560 | 3340 | 1940 | 780 | 1840 | 580 | 4780 | 1620 | 560 |
| *Acremonium rutilum* | 800 | 2000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 80 | 0 | 0 | 0 | 0 | 0 |
| *Aspergillus* | 6300 | 600 | 400 | 0 | 220 | 820 | 1560 | 3340 | 1920 | 780 | 1840 | 580 | 4780 | 1620 | 560 |
| *A. aculeatus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 232 | 40 | 0 | 32 | 0 | 244 | 184 | 0 |
| *A. aeneus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 16 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. awamori* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. carbonarius* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 32 | 0 | 0 | 0 | 0 |
| *A. ellepticus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. ficuum* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. flavus* | 5600 | 0 | 400 | 0 | 0 | 720 | 1320 | 0 | 0 | 0 | 240 | 0 | 740 | 0 | 0 |
| *A. flavus* var. *columnaris* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. fotedius* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. fotedius* var. *acidus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. fotedius* var. *pallidus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 24 | 0 | 0 | 0 |
| *A. fumigatus* | 200 | 600 | 0 | 0 | 0 | 80 | 240 | 0 | 100 | 40 | 1160 | 40 | 1860 | 0 | 120 |
| *A. heteromorphus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 120 | 0 | 0 | 0 |
| *A. japonicus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1160 | 60 | 0 | 0 | 100 | 1020 | 700 | 0 |
| *A. niger* | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 0 | 140 | 0 | 0 | 440 |
| *A. oryzae* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1020 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. parasiticus* | 300 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1160 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. phoenicis* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 260 | 0 | 0 | 0 | 0 |
| *A. pluverulentus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. terreus* | 200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 120 | 20 | 0 | 20 | 0 | 0 |
| *A. terreus var. Aureus* | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. tubengensis* | 0 | 0 | 0 | 0 | 120 | 0 | 0 | 0 | 180 | 620 | 0 | 60 | 20 | 0 | 0 |
| *Fusarium oxysporum* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Mucor circinelliodes* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Penicillium lanosum* | 0 | 400 | 200 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Talaromyces* | 1000 | 2200 | 8000 | 20 | 0 | 60 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *T. duclauxii* | 800 | 0 | 7000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *T. luteus* | 0 | 2200 | 1000 | 20 | 0 | 60 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *T. purpurogenus* | 200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Trichoderma viride* | 400 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Verticillium terrestre* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table 3: Continued**

| Samples / Fungal taxa | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Total count** | 80 | 1880 | 1440 | 2460 | 2660 | 4980 | 1260 | 860 | 4160 | 4620 | 880 | 1360 | 620 | 840 | **200** |
| *Acremonium rutilum* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Aspergillus* | 80 | 1880 | 1440 | 2460 | 840 | 4980 | 1160 | 860 | 4160 | 4620 | 880 | 1360 | 620 | 840 | 200 |
| *A. aculeatus* | 0 | 0 | 0 | 40 | 40 | 0 | 0 | 0 | 0 | 40 | 0 | 580 | 0 | 120 | 0 |
| *A. aeneus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. awamori* | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. carbonarius* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 520 | 120 | 0 | 0 | 0 | 0 | 0 |
| *A. ellepticus* | 0 | 1280 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. ficuum* | 0 | 0 | 20 | 60 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 140 |
| *A. flavus* | 0 | 0 | 0 | 0 | 220 | 0 | 360 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. flavus* var. *columnaris* | 0 | 0 | 0 | 2240 | 0 | 3200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. fotedius* | 0 | 0 | 20 | 0 | 0 | 0 | 280 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. fotedius* var. *acidus* | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 |
| *A. fotedius* var. *pallidus* | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 80 | 0 | 0 | 0 | 40 | 0 | 0 | 0 |
| *A. fumigatus* | 80 | 0 | 260 | 80 | 260 | 0 | 280 | 0 | 0 | 580 | 780 | 480 | 480 | 0 | 0 |
| *A. heteromorphus* | 0 | 600 | 0 | 0 | 0 | 0 | 0 | 360 | 460 | 40 | 0 | 0 | 0 | 0 | 0 |
| *A. japonicus* | 0 | 0 | 0 | 0 | 0 | 1780 | 0 | 0 | 0 | 40 | 0 | 0 | 140 | 0 | 0 |
| *A. niger* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 420 | 0 | 0 | 0 | 260 | 0 | 0 | 0 |
| *A. oryzae* | 0 | 0 | 1120 | 0 | 0 | 0 | 0 | 0 | 0 | 2340 | 0 | 0 | 0 | 0 | 0 |
| *A. parasiticus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3180 | 1460 | 0 | 0 | 0 | 640 | 0 |
| *A. phoenicis* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 60 | 40 |
| *A. pluverulentus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 |
| *A. terreus* | 0 | 0 | 0 | 0 | 200 | 0 | 240 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 0 |
| *A. terreus* var. *Aureus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. tubengensis* | 0 | 0 | 0 | 40 | 80 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Fusarium oxysporum* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 80 |
| *Mucor circinelliodes* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Penicillium lanosum* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Talaromyces* | 0 | 0 | 0 | 0 | 1820 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 144 | 0 |
| *T. duclauxii* | 0 | 0 | 0 | 0 | 300 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *T. luteus* | 0 | 0 | 0 | 0 | 1520 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *T. purpurogenus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 720 | 0 |
| *Trichoderma viride* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Verticillium terrestre* | 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 ±2ºC, 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 28ºC 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 coloines/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 specifiera*.

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 inmoderate 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** / **Fungal taxa** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Total count** | **36** | **47** | **21** | **32** | **23** | **36** | **64** | **30** | **17** | **27** | **23** | **17** | **27** | **39** | **24** |
| *Acremonium rutilum* | 0 | 13 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Aspergillus* | 30 | 34 | 21 | 30 | 22 | 36 | 27 | 22 | 17 | 26 | 23 | 17 | 27 | 39 | 23 |
| *A. aculeatus* | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 2 | 4 | 0 | 0 |
| *A. avenaceus* | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. awamori* | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. carbonarius* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 10 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. ellepticus* | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 |
| *A. ficuum* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 4 |
| *A. flavus* | 10 | 11 | 0 | 0 | 0 | 19 | 15 | 0 | 0 | 8 | 0 | 0 | 0 | 19 | 0 |
| *A. flavus* var. *columnaris* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. fotedius* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 |
| *A. fotedius* var. *acidus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. fotedius* var. *pallidus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. fumigates* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 3 | 1 | 7 | 0 | 0 |
| *A. japonicus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 4 | 0 | 2 | 12 | 0 | 0 |
| *A. niger* | 0 | 0 | 0 | 20 | 19 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| *A. oryzae* | 0 | 0 | 19 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 |
| *A. parasiticus* | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. phoenicis* | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 5 | 0 | 0 | 2 | 4 | 0 | 0 | 0 |
| *A. pluverulentus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 4 | 20 | 0 |
| *A. terreus* | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. terreus* var. *aureus* | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. tubengensis* | 8 | 18 | 0 | 0 | 0 | 0 | 0 | 5 | 7 | 14 | 16 | 0 | 0 | 0 | 0 |
| *Curvularia specifiera* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Fusarium oxysporum* | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Mucor circinelliodes* | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Pencicillium nigricans* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Talaromyces* | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| *T. duclauxii* | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| *T. purpurogenus* | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| *Verticillium terrestre* | 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 / Fungal taxa | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Total count** | **16** | **20** | **27** | **29** | **14** | **37** | **21** | **35** | **21** | **21** | **30** | **33** | **26** | **22** | **13** |
| *Acremonium rutilum* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Aspergillus* | 16 | 20 | 24 | 29 | 14 | 37 | 21 | 35 | 21 | 16 | 23 | 31 | 24 | 18 | 8 |
| *A. aculeatus* | 0 | 0 | 0 | 0 | 3 | 18 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *A. avenaceus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. awamori* | 4 | 4 | 11 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| *A. carbonarius* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. ellepticus* | 0 | 8 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. ficuum* | 4 | 0 | 0 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 |
| *A. flavus* | 3 | 0 | 10 | 7 | 0 | 19 | 5 | 11 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| *A. flavus* var. *columnaris* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 |
| *A. fotedius* | 0 | 0 | 0 | 6 | 0 | 0 | 8 | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. fotedius* var. *acidus* | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| *A. fotedius* var. *pallidus* | 0 | 8 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 |
| *A. fumigates* | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| *A. japonicus* | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| *A. niger* | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 8 | 20 | 5 | 0 | 10 | 7 | 2 | 0 |
| *A. oryzae* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| *A. parasiticus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 14 | 0 | 3 | 0 |
| *A. phoenicis* | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 4 | 7 |
| *A. pluverulentus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 |
| *A. terreus* | 1 | 0 | 3 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. terreus* var. *aureus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. tubengensis* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 7 | 0 | 0 | 0 |
| *Curvularia specifiera* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| *Fusarium oxysporum* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Mucor circinelliodes* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| *Penicillium nigricans* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Talaromyces* | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 7 | 1 | 2 | 3 | 1 |
| *T. duclauxii* | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 7 | 1 | 2 | 3 | 0 |
| *T. purpurogenus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| ***Verticillium** terrestre* | 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 speciese 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 terrestreas* 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 28ºC 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 28ºC. 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 speciese 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 frequences of occurrence in both two methods of isolation, as well as *A. niger*, *A. phoenicies* and *A. Tubengencies* 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). Aspergilli 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 bagasseis 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** | | | | |
| *Acremonium rutilum* W. Gams | 2880 | 4.13 | 10.0 | 3 | R | 15 | 1.8 | 10.0 | 3 | R |
| *Aspergillus* | 51700 | 74.15 | 96.7 | 29 | H | 731 | 88.3 | 100 | 30 | H |
| *A. aculeatus* Iizuka | 4220 | 6.05 | 33.3 | 10 | M | 36 | 4.3 | 20.0 | 6 | L |
| *A. aeneus* Sappa | 80 | 0.11 | 3.3 | 1 | R | - | - | - | - | - |
| *A. avenaceus* Smith | - | - | - | - | - | 7 | 0.8 | 3.3 | 1 | R |
| *A. awamori* Nakazawa | 20 | 0.03 | 3.3 | 1 | R | 37 | 4.5 | 20.0 | 6 | L |
| *A. carbonarius* (Bainier) Thom | 800 | 1.15 | 10.0 | 3 | R | 15 | 1.8 | 6.7 | 2 | R |
| *A. ellepticus*, sp.nov. | 1280 | 1.84 | 3.3 | 1 | R | 30 | 3.6 | 13.3 | 4 | L |
| *A. ficuum* (Reich.) Hennings | 220 | 0.32 | 10.0 | 3 | R | 24 | 2.9 | 20.0 | 6 | L |
| *A. flavus* Link | 9600 | 13.77 | 26.7 | 8 | M | 139 | 16.8 | 46.7 | 14 | M |
| *A. flavus* var. *columnaris* Raper & Fennell | 5440 | 7.80 | 6.7 | 2 | R | 9 | 1.1 | 3.3 | 1 | R |
| *A. fotedius* (Naka.) Thom and Raper | 300 | 0.43 | 6.7 | 2 | R | 34 | 4.1 | 13.3 | 4 | R |
| *A. fotedius* var. *acidus* N., S., &W | 120 | 0.17 | 6.7 | 2 | R | 6 | 0.7 | 6.7 | 2 | R |
| *A. fotedius* var. *pallidus* N., S., &W | 300 | 0.43 | 16.7 | 5 | L | 15 | 1.8 | 10.0 | 3 | R |
| *A. fumigatus* Fresenius | 7720 | 11.07 | 63.3 | 19 | H | 18 | 2.2 | 23.3 | 7 | L |
| *A. heteromorphus* Batista and Maia | 1580 | 2.27 | 16.7 | 5 | L | - | - | - | - | - |
| *A. japonicus* Saito | 5000 | 7.17 | 26.7 | 8 | M | 29 | 3.5 | 20.0 | 6 | L |
| *A. niger* V. Tieghem | 1460 | 2.09 | 20.0 | 6 | L | 102 | 12.3 | 36.7 | 11 | M |
| *A. oryzae* (Ahlb.) Cohn | 4480 | 6.43 | 10.0 | 3 | R | 33 | 4 | 13.3 | 4 | L |
| *A. parasiticus* Speare | 6740 | 9.67 | 16.7 | 5 | L | 29 | 3.5 | 13.3 | 4 | L |
| *A. phoenicis* (Corda) Thom | 360 | 0.52 | 10.0 | 3 | R | 40 | 4.8 | 26.7 | 8 | M |
| *A. pluverulentus* (Mc-Alp.) Thom | 20 | 0.03 | 3.3 | 1 | R | 31 | 3.7 | 13.3 | 4 | L |
| *A. terreus* Thom | 820 | 1.18 | 23.3 | 7 | L | 14 | 1.7 | 13.3 | 4 | L |
| *A. terreus* var. *aureus* Thom&Raper | 20 | 0.03 | 3.3 | 1 | R | 2 | 0.2 | 3.3 | 1 | R |
| *A. tubengensis* (Schober) Moss. | 1120 | 1.61 | 23.3 | 7 | L | 81 | 9.8 | 26.7 | 8 | M |
| *Curvularia specifiera* (Bainier) Boedijin | - | - | - | - | - | 1 | 0.1 | 3.3 | 1 | R |
| *Fusarium oxysporum* Schlechtenda | 80 | 0.11 | 3.3 | 1 | R | 7 | 0.8 | 3.3 | 1 | R |
| *Mucor circinelliodes* (Van Tieghem) | 20 | 0.03 | 3.3 | 1 | R | 8 | 1 | 10.0 | 3 | R |
| *Penicillium* Thom&Raper | 620 | 0.89 | 10.0 | 3 | R | 1 | 0.12 | 3.3 | 1 | R |
| *P. lanosum* Westling | 620 | 0.89 | 10.0 | 3 | R | - | - | - | - | - |
| *P. nigricans* (Bainier) Thom | - | - | - | - | - | 1 | 0.1 | 3.3 | 1 | R |
| *Talaromyces* | 13920 | 19.97 | 36.7 | 11 | M | 27 | 3.26 | 40.0 | 12 | M |
| *T. duclauxii* Delacroxi | 8200 | 11.76 | 13.3 | 4 | L | 23 | 2.8 | 26.7 | 8 | M |
| *T. luteus* (Zukal) Benjamin | 4800 | 6.88 | 16.7 | 5 | L | - | - | - | - | - |
| *T. purpurogenus* Stool | 920 | 1.32 | 6.7 | 2 | R | 4 | 0.5 | 13.3 | 4 | L |
| *Trichoderma viride* Pers. ex S.F. Gray | 420 | 0.60 | 6.7 | 2 | R | - | - | - | - | - |
| *Verticillium terrestre* (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 4varieties** | | | | | **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*, *Chaetomidium* and *Trichderma* 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).

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