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Utilization of Agro-industrial wastes as carbon source in solid-state fermentation processes for the production of value-added byproducts

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Abstract

Utilization of agro industrial wastes as solid support was evaluated in solid state fermentation (SSF) methods to produce value-added byproducts such as antioxidants and antimicrobial agents. Among these agricultural wastes added as a carbon source in SSF process are corn cobs, olive mill, wheat bran, rice straw, rice bran and sorghum. The biological activities of the extracts of ethyl acetate (EtOAc) of *Aspergillus pseudocaelatus* MG772677 and *Trichoderma gamsii* KX685665 strains were studied. The extract of ethyl acetate of *A. pseudocaelatus* cultured on medium containing sorghum as natural carbon source showed the highest inhibitory activity against tested microorganisms. The antioxidant activity results varied based on the used waste as a carbon source as well as the incubation period. The EtOAc extracts of *A. pseudocaelatus* and *T. gamsii* cultivated on different agricultural wastes media showed potent antioxidant activities of scavenging DPPH when compared to those cultivated on PDA media. The highest percentage of antioxidant activity (53.84%) for the EtOAc extract of *T. gamsii* was observed after 7 days of incubation period.

Keywords: Agro-industrial wastes *Aspergillus pseudocaelatus* MG772677, *Trichoderma gamsii* KX685665, Antimicrobial, Antioxidant and solid-state fermentation

1. Introduction

The manufacturing processing of agricultural raw materials generates a huge amount of wastes which are either burned or used for animal feeding. These wastes are usually rich in carbohydrates, proteins and minerals, and should be exploited as raw materials for other industries. Accumulation of agricultural wastes in huge quantities annually results not only in the environmental deterioration, but also in economic loss as these wastes can be utilized for production of high valuable products, for instance food, energy and chemicals producing (Singh, 2009). The carbon content, nutrient and moisture in these agricultural wastes offer favorable environment for the microbial growth, which opens up considerable potential for recycle in solid state fermentation (Mussato *et al.*, 2012).

The microbial species can exploit these collective wastes from agricultural industries, specially, fungi strains, which have the ability to produce hydrolytic enzymes such as chitinase and cellulose to ferment these wastes. The utilization of industrial agricultural waste in solid state fermentation is of particular importance because it is considered available and economic, as well as being an eco-friendly alternate for their discarding. The fermentation could be affected by some factors which could maximize the produced yields; these factors include the modifying of substrates size, moisture content, pH, temperature… etc. (Nigam and Pandey, 2009). In general, produced yield can be maximized by selecting an appropriate substrate or a combination of substrates with suitable conditions (Mussatto *et al.,* 2012). The large scale production of secondary metabolites requires studying the effective factors which include abiotic and biotic factors (Shentu *et al.*, 2013).

Fungi have been proven to be potent source for biologically active compounds with therapeutic potential (Hoeksma *et al.,* 2019) and has produced a number of compounds of medical importance, including penicillin, Lovastatin and caspofungin (Goler 2007; Keating and Figgitt, 2003; Vandermolen *et al.,* 2013). *Aspergillu*s and *Trichoderma* species are a valuable source of commercial enzymes used in the recycling of cellulose waste (Reino and Guerrero, 2008).

New and better techniques for the recovery of agricultural waste have been developed due to industrial innovation and high technology. These techniques contribute to maintaining resources efficiency, sustainable production, consumption and reduction of negative environmental impacts (Duque-Acevedo *et al.,* 2020). The aim of this research was to study the application of various industrial agricultural wastes as a carbon source or nutrient

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in SSF processes in order to produce antimicrobials and antioxidant agents.

2. Materials and Methods

2.1. Fungal strains

Two fungal strains, *Aspergillus pseudocaelatus* and *Trichoderma gamsii*, were recovered from rhizosphere area of medicinal plants (*Aloe vera*), Basil, (*Ocimum basilicum*), and Peppermint (*Mentha piperita*), planted in Sekem farm, Heliopolis University, Cairo, Egypt under organic farming regulations. The strains were maintained and stored on the surface of PDA slants at 4° C. They were identified on the basis of their morphological and microscopic characteristics as well as 18s rDNA (Zohair *et al.*, 2018).

2.2. Solid State Fermentation

Corncobs, olive mill pomace, rice bran, rice straw, sorghum and wheat bran wastes were obtained from the local fields in Giza governorate, Egypt, dried in oven at 55 °C for 48 h and ground to 40-mesh (400 um) were utilized as fermentation carbon sources. Three grams of the solid substrates were added to Erlenmeyer flasks (250 ml). The substrate moisture content was adjusted to 75% with solution of minerals (KH_2PO_4; 2.0 g/L, $CaCl_2$; 0.3 g/L, $MgSO_4$; 0.3 g/L, $FeSO_4$;0.11 g/L, $ZnSO_4$; 0.3 g/L). The pH of prepared solutions was adjusted to 6 then added to the solid substrate. The flasks contents were mixed well and autoclaved for 20 min at 121 ºC at 1 atm and then inoculated individually with 2discs each 6mm in diameter of *A. pseudocaelatus* or *T. gamsii* fungal strains grown on PDA fresh cultures. Incubation was carried out at 25-28 ºC up to 19 days. Flasks, incubated without inoculation, were used as a negative control, while flasks containing 100 ml of PDA medium inoculated with strains *A. pseudocaelatus* or *T. gamsii* strains were used as positive control.

2.3. Liquid culture and metabolite production

2.3.1. Extraction of crude extract

After different incubation period (7,11,15 and 19 days) cultivation medium and mycelial mats were soaked overnight in ethyl acetate (1:1, v/v), then were homogenized in ethyl acetate using an Ultra Sonic wave (*J.P selecta s.a* sonicator). The extraction process was carried out three times for complete extraction. All of the organic fractions were collected and dried using rotary evaporator at 40°C (180 rpm) under vacuum condition. The dried crude extract was subjected to biological activity evaluation (Zohair *et al.*, 2018).

2.3.2. Biological activity of the isolated fungal secondary metabolites

A. pseudocaelatus and *T. gamsii* ethyl acetate extracts were tested biologically using different bio-assays to determine antimicrobial activity using method of agar disc diffusion (El-Sawy *et al*., 2015; Yasser *et al.*, 2020) and antioxidant activity using DPPH assay (Hamed, 2009).

2.3.2.1. Antimicrobial activity

The antibacterial and antifungal activities were screened against different pathogenic strains through the agar disk diffusion methods (El-Sawy *et al*., 2015). Concentrations (1 mg/*5 µl*) of *A. pseudocaelatus* and *T. gamsii* ethyl acetate extracts were assessed against Gram positive bacteria such as *Bacillus subtilis* ATCC6633 and *Staphylococcus aureus* ATCC29213. It was also tested against Gram negative bacteria such as *Escherichia coli* ATCC25922, and *Salmonella enterica* ATCC25566. Also, it was tested against tested yeasts and fungal strains such as *Fusarium solani* NRC15 and *Aspergillus niger* NRC23, in addition to yeasts (*Candida albicans, Candida. trobicalis*). The concentration of tested fungi was 1×10^8 spores/ml. The concentration of the tested bacterial pathogens was 1×10^6 cfu/ml. The test was done within sterilized Petri dishes including 25 ml of sterilized PDA medium in case of fungi and in nutrient agar medium in case of bacteria. Thiophenicol and Treflucan antibiotics were used as positive controls for bacteria and fungi with concentration of 100 µg/disk. The Dimethyl sulfoxide antibiotic (DMSO) was used as a negative control. The prepared disks were loaded on Petri dishes containing the inoculated media then incubated for 24 hours at 30 °C for bacterial strains and 72 hours at 28 °C for fungal strains, respectively. The inhibition zones were measured.

2.3.2.2. In vitro determination of antioxidant activity

This assay was performed according to Hamed (2009) with some modifications. One mg of ethyl acetate fungal extract was dissolved in 1 ml of Dimethyl sulfoxide (DMSO) to prepare 1000 µg/ml stock solution. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (0.004 mg) was dissolved in 100 ml of methyl alcohol HPLC grade to make concentration 0.004% solution. Different concentrations (5-25 µg) of reference standard compounds such as Quercetin and Vitamin C were prepared. In a 96-wellplate, 20 µl of stock solutions (samples –or standard) was added into each well then 180 µl of methanol solution of DPPH (0.004%) was added to complete the final concentration of evaluated samples 100 µg/ml. After 30 min. of incubation, the plate was scanned at λ = 540 nm using microplate reader. In case of blank, 20 µl of dissolving agent (DMSO) were added instead of 20 µl of samples. The assay was repeated twice to confirm the records. The activity of radical scavenging could be determined based on the given equation:

Scavenging Activity (%) = $[(A_{Blank} - A_{Sample}) / A_{blank}] \times 100$

Where, A_{Blank} (Absorbance of mixture without sample "DPPH only"), A_{Sample} (Absorbance of test samples).

Samples that indicated 50% or higher antioxidant activity at 100 µg / ml concentration compared to control were considered active. The effect of scavenging on DPPH free radicals was estimated according to (Shimada *et al.*, 1992).

2.4. Data Analysis

The analysis of variance (ANOVA) was evaluated using MSTATC software. The differences of significance between means were compared based on the least significance differences (LSD) test at 5% significant level.

3. Results and Discussion

3.1. Activity of the fungal extract as Antimicrobial agents

A. pseudocaelatus EtOAc extract showed a strong antimicrobial activity as shown in (Table 1). It presented a

strong inhibitory activity against pathogenic tested yeasts and fungal strains (*Candida albicans, C. trobicalis, Fusarium solani, Rhizoctonia solani, Sclerotium rolfsii and Verticillium dahliae*) with zones of inhibition 13.5, 15.5, 14, 15, 7 and 8 mm diameter, respectively. For bacterial tested strains, *A. pseudocaelatus* extract has a significant effect against *B.subtilis* ATCC 6633, *E.coli* ATCC 25922, *S. aureus* ATCC 25923 and *S. enteric* ATCC 25566 with inhibition zones of 20, 19.5, 18.5 and 14.5 mm, respectively.

Table 1. Antimicrobial effect of *A. pseudocaelatus* extract.

Pathogenic		*A. pseudocaelatus* extract	Positive control
		Diameter of inhibition zone (mm)	
G+ve bacterial strains	*B.subtilis*	20±0.0	22.5±0.5
	S.aureus	13.5±0.5	18.5±0.5
G-ve bacterial strains	*E.coli*	18.5±0.5	11.5±0.5
	S.enterica	14.5±1.5	15±0.0
Yeast	*C.albicans*	13.5±0.5	7.5±0.5
	C.tropicalis	15.5±0.5	7±0.0
Fungi	*F.solani*	14±0.6	7±0.0
	R.solani	15±0.0	7±0.0
	S.rolfsii	7±0.0	7±0.0
	V.dahliae	8±.01	8.5±0.5

Notes: The inhibition zone measured as diameter expressed in (mm). (Values are mean ± S.D.)

Treflucan and Thiophenicol were applied with a concentration of 100 µg/disc as positive controls, DMSO was applied as negative control.

Extract of *T. gamsii* fungus resulted in a moderate activity against all yeasts and fungal pathogenic tested pathogens strains (*C.albicans*, *C.tropicalis*, *F.solani*, *R.solani*, *S.rolfsii* and *V.dahliae*) with inhibition zones of 8, 7, 8, 7, 7 and 7mm diameter, respectively. In parallel, the extract of *T. gamsii* had a moderate effect against *B. subtilis* ATCC6633, *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *S. enteric* ATCC 25566 with diameter of inhibition zone 12, 13.5, 9 & 7 mm, respectively as shown in (Table 2**).**

Table 2. Antimicrobial effect of *T. gamsii* extract.

Pathogenic		*T. gamsii* extract	Positive control
		Diameter of inhibition zone (mm)	
G+ve bacterial strains	*B.subtilis*	12±0.0	22.5±0.5
	S.aureus	13.5±0.5	18.5±0.5
G-ve bacterial strains	*E.coli*	9±0.0	11.5±0.5
	S.enterica	7±0.0	15±0.0
Yeast	*C.albicans*	8±0.0	7.5±0.5
	C.tropicalis	7±1	7±0.0
Fungi	*F.solani*	8±0.0	7±0.0
	R.solani	7±0.0	7±0.0
	S.rolfsii	7±0.0	7±0.0
	V.dahliae	7±0.0	8.5±0.5

Notes: The inhibition zone measured as diameter expressed in (mm). (Values are mean ± S.D.)

Treflucan and Thiophenicol were applied with a concentration of 100 µg/disc as positive controls, DMSO was applied as negative control.

Saleh *et al.* (2011) found that some of *Trichoderma* spp crude extract had an antibacterial effect against some bacterial pathogens with zones of inhibition fluctuating from (10 to 28 mm). Also**,** Vinale *et al.* (2006) indicated that *Trichoderma harzianum* fungal strains (T22 &T39) secondary metabolites showed an inhibition activity against plant pathogens *Pythium ultimum* and *R.solani*. From the above results, the *A. pseudocaelatus* ethyl acetate extract was more effective than the *T. gamsii* extract. These results were in agreement with Zohair *et al.*, (2018) who indicated that the biological activity of EtOAc extract of *A. pseudocaelatus* was more active than that of *T. gamsii*. It showed an effective inhibitory result against fungal test strains (*C.albicans, C.tropicalis, F.solani, R.solani, S.rolfsii & V.dahliae*).

Both fungi showed the capability of utilization of different agricultural industrial wastes used as carbon sources. As shown in Fig.1.

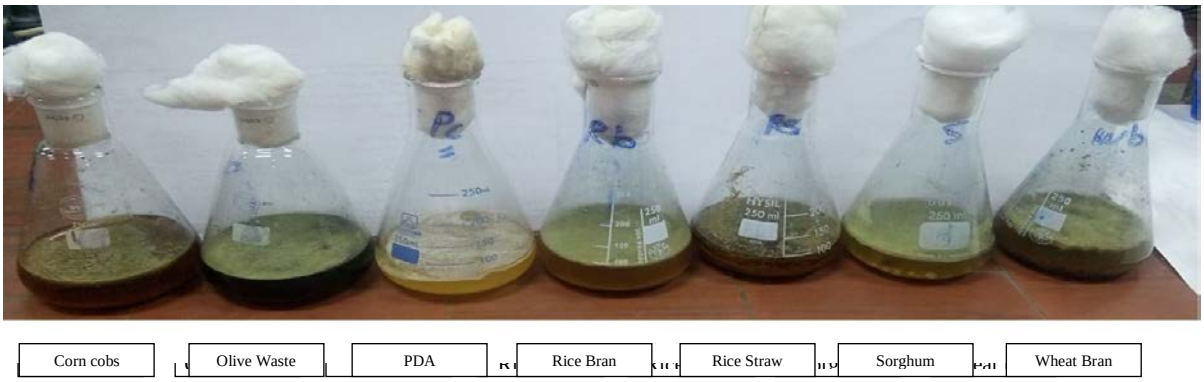

Figure 1. *A. pseudocaelatus* cultivated on media contain different agricultural industrial wastes as carbon sources

The most effective one as a medium was the agriculture waste, sorghum. The highest inhibition zone was obtained from *A. pseudocaelatus* cultivated on media containing sorghum as carbon sources after 15 days of incubation period. It exhibited antibacterial activity against both Gram-positive bacterial strains *B.subtilis* ATCC6633 and *S.aureus* ATCC25923 with inhibition zones of 26.0 and 22.5 mm diameter, respectively. Also, it exhibited antibacterial activity against Gram-negative bacterial strains *E. coli* ATCC25922 and *S.enterica* ATCC25566 with inhibition zones of 24.0 and 22.5 mm diameter, respectively as shown in (**Fig. 2**).

Many studies have suggested that sorghum and sorghum processing waste have a huge potential for the production of value added products due to its content of fermentable sugars (starch, sucrose, glucose and fructose),

and lignocelluloses feed stock. Makanjuola *et al.* (2019) reported that the high residual starch (up to 53% (w/w) in sorghum waste makes it rich medium for *Aspergillus awamori* growth for production of value added product.

The anti-yeast activity was 22.5 and 13.0 mm with *Candida albicans* ATCC 10321 and *Candida tropicalis* ATCC750, respectively, while the antifungal activity was 21.0 and 20.0 mm against *F. oxysporium* and *A. niger* as presented in (Fig. 2 & 3).

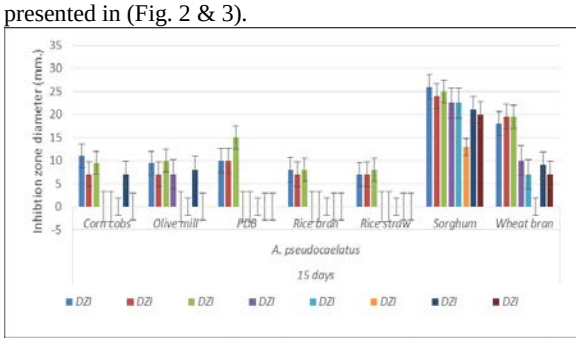

Figure 2. Antimicrobial effect of ethyl acetate fungal extract of *A. pseudocaelatus* using solid state fermentation after 15 days of incubation period.

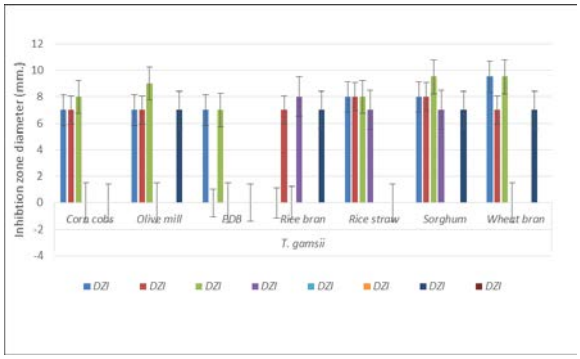

Figure 3. Antimicrobial effect of ethyl acetate fungal extract of *T. gamsii* using solid state fermentation after 15 days of incubation period.

Pandey *et al.* (2001) reported production of Cyclosporin A from *Tolypocladium infautum* using wheat bran and oxytetracycline antibiotic by *S. rimosus* using corn cob in solid state fermentation.

Ethyl acetate extract of *A. pseudocaelatus* on media containing sorghum as natural alternative carbon sources had the higher inhibitory activity against all tested microorganism than using PDA media. It showed antibacterial activity against both Gram-positive bacteria (*B. subtilis* ATCC6633 and *S. aureus* ATCC25923) and Gram-negative bacteria (*E.coli* ATCC25922 and *S. enterica* ATCC25566) with inhibition zones of 26.0, 22.5, 24.0 and 22.5 mm, respectively while the inhibition zone of EtOAc extract of *A. pseudocaelatus* growing on PDA media was 20, 19.5, 18.5 and 14.5 mm, against *B. subtilis* ATCC 6633, *S. aureus* ATCC 25923, *E.coli* ATCC 25922 and *S. enteric* 25566, respectively.

Also, the anti-pathogenic yeast activity was 22.5 and 13.0 mm with *C. albicans* ATCC 10321 and *C. tropicalis* ATCC 750, respectively using the sorghum as natural alternative carbon sources compared with 13.5 and 15.5mm diameter of ethyl acetate extract of *A. pseudocaelatus* fermented on PDA for 15 days of incubation**.**

3.2. In vitro antioxidant activity of ethyl acetate extract

The antioxidant capacity of the fungal isolates is shown in **Table 3**. The DPPH scavenging activity varied between the two fungal isolates ethyl acetate extracts. The *T. gamsii* extract showed a higher antioxidant activity than *A. pseudocaelatus extract.*

Table 3. Antioxidant potential of ethyl acetate (EtOAc) extract of *A. pseudocaelatus* and *T. gamsii* cultivated on PDA media

Ethyl acetate extract	Antioxidant activity %
A. pseudocaelatus	1.926
T. gamsii	14.788

The variation on the antioxidant activity can be attributed to the used waste as a carbon source and the incubation period. Both isolates showed differential utilization of the various carbon sources. The use of corn cobs waste as a carbon source enhanced the optimization of antioxidant activity percentage. The highest percentage of antioxidant activity observed was 53.84% for *T. gamsii* ethyl acetate extract after 7 days of incubation **(Table 4).**

Table 4. *In vitro* antioxidant activity (%) of *A. pseudocaelatus* and *T. gamsii* ethyl acetate extract cultivated on media containing different agricultural wastes and their different incubation periods (days).

Waste/ Incubation Period	7 days	11 days	14 days	19 days
	Ethyl acetate extract of *A. pseudocaelatus*			
Corn cobs	27.051	28.049	18.136	31.339
Olive meal	4.379	3.681	7.254	6.411
PDB	27.217	23.736	18.080	16.142
Rice bran	4.780	11.154	6.250	3.492
Rice straw	6.648	7.422	15.179	21.294
Sorghum	4.286	16.853	9.845	9.559
Wheat bran	4.478	18.080	8.930	9.134
	Ethyl acetate extract of *T. gamsii*			
Corn cobs	53.846	27.679	21.694	31.241
Olive meal	10.165	2.679	1.202	1.577
PDB	7.923	6.585	5.209	19.191
Rice bran	5.989	13.728	0.801	2.231
Rice straw	31.703	5.636	9.960	8.539
Sorghum	4.945	6.250	4.236	10.800
Wheat bran	7.418	14.286	6.926	9.015

The antioxidant effects of the *A. pseudocaelatus* and *T. gamsii* extracts ranged from 3.681 to 31.33% for olive waste and corncobs after 11 and 19 days incubation times, respectively. Meanwhile it ranged 1.20 and 53.84 % of DPPH scavenging, respectively with *A. pseudocaelatus and T. gamsii* cultivated on olive mill and corncobs after 14 days and 7 days, respectively. The fungal extracts of *A. pseudocaelatus* and *T. gamsii* cultivated on PDA showed antioxidant activity of 1.92 and 14.78 %, respectively.

Awad *et al.* (2018) reported that the volatile compound extracted from *Trichoderma viride* mycelia showed antioxidant effects by 29.62%, 63.12% and 70.37% at concentrations of 10, 50 and 100 µg, respectively. Moreover, other constituents such as carbohydrates, proteins could have remarkable antioxidant effects ranging from 3.70 - 33.00% for proteins and 3.00 - 23.00% for carbohydrates at concentrations of 10 - 100 µg, respectively.

The ability of the used fungal strains to produce hydrolytic enzymes could offer a promising chance for

exploiting these strains in waste conversion. Zohair *et al.* (2018) reported that *A. pseudocaelatus* exhibited a high hydrolytic ability to utilize carboxy methylcellulose and a moderate hydrolytic capacity of chitin (62.14 and 34.29 %), respectively, while *T. gamsii* had a high ability for hydrolyzing of carboxymethyl cellulose (CMC) and chitin 70.00 and 36.25 %, respectively. The use of hydrolytic enzymes to degrade agricultural wastes to fermentable sugar is highly recommended due to the specific nature of the enzymes and their ability to work at mild process conditions. The saccharification of lignocellulosic material requires the use of several enzymes with complementary activities as the endoglucanase, which attacks regions in the interior of linear cellulose chains, the exoglucanases or cellobiohydrolases, which hydrolyze cellobiose units from the ends of cellulose chains and β-glucosidase, which converts cello-oligosaccharides and cellobiose into glucose (Stichnothe *et al.*, 2016)

4. Conclusion

The ethyl acetate extract of *A. pseudocaelatus* cultured on medium containing sorghum as natural carbon sources showed higher inhibitory effect against the tested pathogenic microorganism than using PDA medium. In the same way, *T. gamsii* ethyl acetate extract of PDA medium showed the highest percentage of antioxidant activity 53.84% after 7 days of incubation period compared to 14.7 %. Exploiting of agro-industrial waste for the production of sustainable bio-resources and the conversion of these resources and wastes into valuable products should be initiated by implementing the biotechnological innovation perspective to establish economic sustainability

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Conflict of interest

The authors declare that there is no conflict of interest.

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