

Gibberellic Acid Production by *Fusarium moniliforme* and *Aspergillus niger* Using Submerged Fermentation of Banana Peel

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Abstract

The present study aimed to produce gibberellic acid through fermentation using banana (*Musa sapientum*) peel waste as substrate. Banana peel, a domestic and industrial waste, constitutes a potential source of cheap fermentable substrate for the production of other value-added products. *Fusarium moniliforme* ATCC 10052 and *Aspergillus niger* CBS 513.88 were used as fermenting organisms. The substrate was dried, ground and its proximate composition determined. The powdered substrate was added to a modified CzapekDox broth (a semisynthetic medium), with Carboxyl methylcellulose (CMC) as control. The fermentation conditions were: pH 5.5; inoculum size 1% (5×10^5 spores/mL *F. moniliforme*) (2×10^6 spores/mL *A. niger*); substrate concentration 2 g; temperature 25 ± 2 °C; fermentation time 7 days. The fermentation was optimized by varying pH, inoculum size, substrate concentration and fermentation time. The extracted GA was subjected to infra-red spectroscopy using FT-IR. The parameters which gave the highest GA yields were thereafter combined in a single fermentation. The results of proximate analysis of banana peel substrate revealed 8.65% moisture, 9.54% protein, 5.40% lipids, 11.45% ash, 22.34% crude fibre, and 42.62% carbohydrate. The GA yields of 13.55 g/L and 12.44 g/L were produced from the banana peel substrate and 3.62 and 2.61 g/L from the CMC control by *F. moniliforme* and *A. niger* respectively. Under optimized conditions, *F. moniliforme* produced 17.48 g/L GA, while *A. niger* produced 13.50 g/L. Extracted GA was similar to standard GA sample and the present results support the potential use of banana peel for fermentative GA production.

Keywords: *Aspergillus niger*; banana peel; fermentation; *Fusarium moniliforme*; gibberellic acid

Abbreviations: BP: Banana Peel; CMC: Carboxymethyl cellulose; GA: Gibberellic acid

Introduction

The gibberellins, defined as a group of naturally occurring plant hormones containing the tetracyclic system, are well-known phytohormones (Bömke and Tudzynski, 2009). Among the gibberellins, the most predominant is gibberellic acid (gibberellin A3 or GA), because of its frequency and high-level occurrence in microbial fermentations as well as its high biological activity in plants (Rademacher, 2016). The gibberellic acid is a naturally occurring plant growth regulator which may produce a variety of effects including stem elongation, sex expression, enzyme induction, leaf and fruit senescence, growth regulation, seed germination and flowering (Rangaswamy, 2012; Kwon and Paek, 2016).

Due to the important characteristics displayed by gibberellic acid, it has found wide applications in agriculture, tissue cultures and in the brewing industry (Ates *et al.*, 2006; Da Silva *et al.*, 2013). In addition, GA has also been applied in activities relating to scientific research

pursuits and pharmacological purposes (Kumar and Lonsane, 1989). GA has an annual global production estimated at 25 tonnes and a global market of about US\$ 500 million per year and price about \$25 /g (Avinash *et al.*, 2003; Rademacher, 2016). There are three reported ways of producing GA which are: plant extraction, chemical synthesis and microbial fermentation, the latter being the most common method due to the very low amounts of GA produced by plants and the costly undertakings of chemical synthesis (Mander, 2003; Sleem, 2013). In addition, chemical synthesis of GA may lead to the production of large amount of toxic effluents with potentially lethal effects to the environment (Dzikowitzky and Schwarzbauer, 2013). Microbial production of GA in a cost-effective manner is an important factor which is considered in industrial processes, and this may be achieved by utilization of agro waste materials (Pandey *et al.*, 2000).

Generally, agro-residues and forest products are considered the best sources of cheap substrates (Nandini *et al.*, 2014).

The fruit selected for use in the current study produces wastes that are available in bulk. The world production of banana was estimated at 99 million tonnes, although this figure is an approximation because a large percentage of the world's banana production comes from subsistence farming on relatively small plots of land and gardens where statistics are lacking (FAO, 2008). Alongside with the large production of the fruit comes the waste disposal problem. The disposal of banana peel is a matter of concern for fruit-processing industries (Padam *et al.*, 2014). Emphasis is laid only on banana fruits harnessed and marketed fresh or as processed juice, while fruit peel produced in great quantities during the process are mainly discarded as waste (Ezeji for *et al.*, 2011). The method of disposal can constitute an environmental or ecological hazard. Instead of discarding these fruit wastes, they can be utilized as cheap substrate for fermentation purposes, where they will serve for the production of other value added products. The microbial production of GA using cheap fermentable substrates has been investigated by various researchers. Several substrates and fermenting organisms that have been used included: wheat bran using *G. fujikuroi* (Kumar and Lonsane, 1990); citric pulp, soy bran, sugarcane bagasse, soy husk, cassava bagasse and coffee husk using *F. fujikuroi* (Rodrigues *et al.*, 2009); municipal sewage sludge using *G. fujikuroi* (Cuali-Alvarez *et al.*, 2011); shea nut shell (*Vitellaria paradoxa*) using *F. moniliforme* (Kobomoje *et al.*, 2013) and *Jatropha curcas* seedcake using *Aspergillus niger* and *A. terreus* (Omojasola and Benu, 2016).

To the best of our knowledge, there is a lack of data on suitability of banana peel in the production of GA. The study, therefore, aimed to establish a dual importance: production of an economically important compound by utilizing readily available agro wastes (whose accumulation could be a burden on the environment) and offering a sustainable, viable and relatively cheap alternative to synthetic GA.

Materials and Methods

Collection of samples and test organisms

Bananas (*Musa sapientum*) were procured from Iyata Market, Ilorin, Kwara State, Nigeria (with coordinates 8.99897 N, 4.561369 E) in the month of November 2016. The banana fruits were authenticated at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, with voucher specimen number UILH/002/1249. The microorganisms used for the fermentation were *Fusarium moniliforme* ATCC 10052 and *Aspergillus niger* CBS 513.88 obtained from the Microbial Culture Collection of the Dept. of Microbiology, University of Ilorin, Nigeria and they were maintained on PDA slants at 4 °C until use.

Substrate preparation

The banana fruits were washed with clean water after which they were peeled. The peel was air-dried at room temperature for 7 days. It was thereafter ground with an electric blender (Binatone BLG 699) to form a fine powder and then stored in a cool and dry place to avoid uptake of moisture (Nandini *et al.*, 2014).

Proximate analysis

The proximate analysis of the substrate was carried out using standard procedures. The parameters investigated were moisture content (Bradley, 2010), lipid, crude fibre, ash, crude protein and carbohydrate contents (AOAC, 1990, 2002).

Spore suspension

The fungal spore inoculum was produced by washing spores of a fully-sporulated (7-day old) Potato Dextrose Agar slant of each test fungus in sterile distilled water (Omojasola and Benu, 2016) and adjusting approximately to 5.0×10^5 spores/mL and 2.0×10^6 spores/mL for *F. moniliforme* and *A. niger* respectively. The size of the inoculum was determined by counting using the improved Neubauer hemocytometer (Weber Scientific International, England).

Fermentation media

The fermentation medium was a modified CzapekDox (Difco) broth using the method of Rangaswamy (2012) with replacement of sucrose with banana peel. The fermentation medium was compounded by adding 2 g of substrate to 100 mL of mineral salts medium. The composition of the mineral salts in 1 L of water was NaNO₃ (3 g), K₂HPO₄ (1 g), MgSO₄·7H₂O (0.5 g), KCl (0.5 g) and FeSO₄ (0.01 g).

Submerged fermentation

The test organisms were drawn separately from the spore suspension and each inoculated into 100 mL of sterile fermenting medium. The fermentation was carried out at 25 ± 2 °C on a rotary shaker (LH Fermentation, Model Mk V orbital shaker) at 150 rpm for 7 days. The final pH was adjusted using 2M NaOH or 1M HCl. Gibberellic acid production was monitored every 24 h.

Optimization of gibberellic acid production

The optimization experiments were conducted varying the following parameters: fermentation period (fermentation was allowed to continue till GA began to drop); pH (4.5 - 5.5); inoculum size (1.0 - 2.0%); substrate concentration (1.0 - 3.0 g).

Media supplementation

The effect of copper sulphate (CuSO₄) supplementation on GA production was evaluated. Three concentrations of CuSO₄ (0.02% w/v, 0.05% w/v and 0.08% w/v) were added to different fermentation media (Chinedu *et al.*, 2010) and fermentation proceeded under the same conditions as the non-supplemented cultures.

Assay of gibberellic acid

The GA was estimated in the supernatant of fermentation media spectrophotometrically (Searchtech 752N UV-VIS) using a modified method described by Berrios *et al.* (2004) at 254 nm. The amount of GA was calculated from the standard curve obtained by dissolving 0.4 g in absolute alcohol and diluted to 100 mL in a volumetric flask with absolute alcohol. Each series of data

obtained from spectrophotometric measurement was fitted by linear regression analysis using GraphPad Prism software. The calibration graph obtained was used for the determination of the concentration of GA with interpolated values after the figures of absorbance obtained were entered.

Recovery of GA

The GA was recovered from the fermentation media using methods described by Rachev *et al.* (1993) and Ates (2006). The fermentation broth was filtered to separate the mycelia from the media. The filtrate was then adjusted to pH 2-2.5 with 2 N HCl and extracted with ethyl acetate (ratio 1:3, filtrate to solvent). The ethyl acetate phase was treated with activated charcoal 1:1.33% (w/v) and re-filtered to remove the activated charcoal. The ethyl acetate phase was extracted with equal volume of saturated NaHCO₃ to separate the GA from other organic impurities. This was further acidified to pH 2.5 with 2 N HCl; re-extracted, dried over anhydrous Na₂SO₄ and concentrated to about 2% of its initial volume using a rotary evaporator. The concentrate was kept at 8 °C for crystallisation.

Infra-red spectroscopy (FT-IR) of recovered GA

Infra-red spectroscopy using FT-IR was used to identify the extracted gibberellic acid by comparing it with the standard spectrum of gibberellic acid (Kem Light Laboratories, India) using the method described by Silverstein *et al.* (2014). The transmittance was carried out in the form of potassium bromate (KBr) pellets in the range of 400-4,000 cm⁻¹ (Thermo Scientific Nicolet iS5 FT-IR).

Statistical analysis

Statistical significance was determined using one-way analysis of variance (ANOVA) and two-way ANOVA, while multiple comparisons between means were determined by Tukey's or Sidak's multiple comparisons test. Analysis was performed using GraphPad Prism software (GraphPad Software Inc. La Jolla, CA, USA) and SigmaPlot for Windows version 10.0 (SysStatSoftwares Inc.). All data are expressed as means of triplicates ± SEM or SD and values of *p* < 0.05 were considered significant, where 'n' represents independent experiments.

Results and Discussion

The data from the hereby study support the suitability of BP (banana peel) as a suitable substrate for the production of GA using *F. moniliforme* and *A. niger* as fermenting organisms. The BP substrate gave higher GA yields than the CMC control (Table 1). The proximate composition of the BP substrate revealed 8.65% moisture, 9.54% protein, 5.40% lipid, 11.45% ash, 22.34% crude fibre and 42.62% carbohydrate. These serve as nutrients for the growth of the fermenting organisms. In addition, the substrate is rich in carbohydrates, which are a valuable raw material that is converted to sugars for the production of GA.

Gibberellic acid production by *F. moniliforme* peaked at 13.55 g/L on day 6, while the maximum yield was 12.44 g/L by *A. niger* (Table 1). In both cases, CMC produced significantly (*p* ≤ 0.05) lower yield than the BP substrate.

Whereas the fermentation conditions varied, the maximum GA production was observed on day 6 and day 4 by *F. moniliforme* and *A. niger* respectively after which a drop in GA yield was recorded. Whereas the pH of the fermentation varied, the maximum GA yield of 13.54 g/L was observed at pH 5.0 by *F. moniliforme* and 12.44 g/L at pH 5.5 by *A. niger*.

The maximum yield of 18.17 g/L by *F. moniliforme* and 13.81 g/L by *A. niger* were recorded on day 4 with 2% inoculum size. The highest GA yield of 13.54 g/L was produced with 2 g substrate concentration by *F. moniliforme* with BP, while the highest yield by *A. niger* was 10.78 g/L at 3 g substrate concentration. The results of CuSO₄ supplementation showed no increase in GA yield by both test organisms (Table 2). The highest yield by *F. moniliforme* was 10.78 g/L on day 6 with 0.08% CuSO₄ supplementation, and 9.27 g/L on day 4 with 0.005% CuSO₄ by *A. niger*. However, no increase in GA yield was recorded with CuSO₄ supplementation of the BP substrate.

The fermentation parameters which gave the highest GA yields were combined in single optimization fermentation. Maximum yields of 17.48 g/L and 13.50 g/L were produced by *F. moniliforme* and *A. niger* respectively.

Table 1. Production of gibberellic acid by submerged fermentation of banana peel using *Fusarium moniliforme* and *Aspergillus niger*

Time (Days)	Gibberellic acid (g/L)			
	<i>Fusarium moniliforme</i>		<i>Aspergillus niger</i>	
	Banana peel	CMC (Control)	Banana peel	CMC (Control)
1	1.85 ± 0.16 ^a	0.14 ± 0.01 ^a	1.74 ± 0.59 ^a	0.03 ± 0.01 ^b
2	2.12 ± 0.08 ^a	0.91 ± 0.02 ^a	3.25 ± 0.18 ^a	0.15 ± 0.01 ^b
3	3.02 ± 0.97 ^a	1.73 ± 0.01 ^a	10.25 ± 0.65 ^a	0.82 ± 0.02 ^b
4	10.04 ± 0.02 ^a	3.62 ± 0.01 ^b	12.44 ± 0.27 ^a	1.24 ± 0.11 ^b
5	11.41 ± 1.37 ^a	1.88 ± 0.04 ^b	3.54 ± 0.03 ^a	2.61 ± 0.07 ^b
6	13.55 ± 0.27 ^a	1.78 ± 0.02 ^b	4.31 ± 0.01 ^a	2.54 ± 0.01 ^b
7	8.67 ± 1.33 ^a	2.63 ± 0.03 ^b	5.38 ± 0.19 ^a	1.46 ± 0.03 ^b

Values represented are means of triplicates ± SEM of amount of gibberellic acid. Means with the same superscript in a column are not statistically different from each other (*F. moniliforme* and *A. niger* were compared separately).

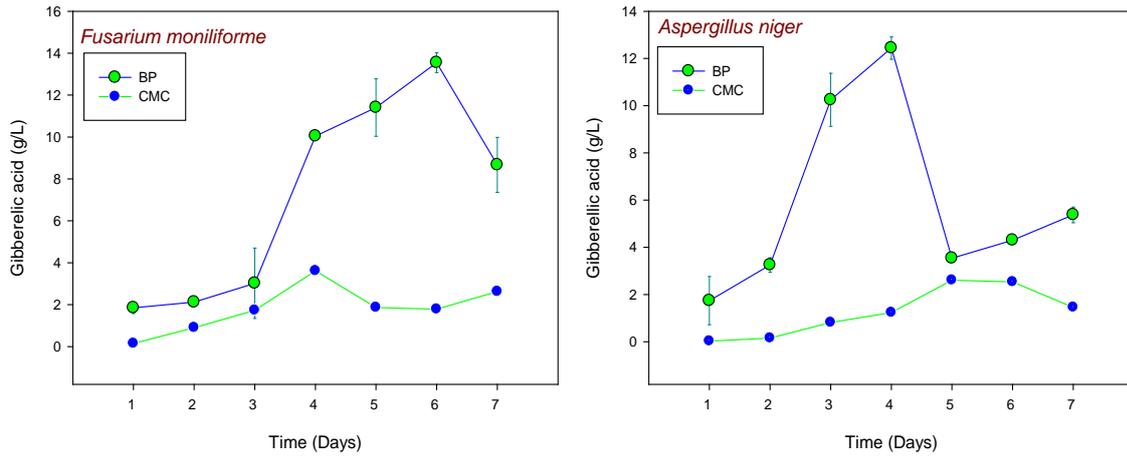


Fig. 1. Effect of varying fermentation time on gibberellic acid production by *F. moniliforme* and *A. niger* using banana peel as substrate

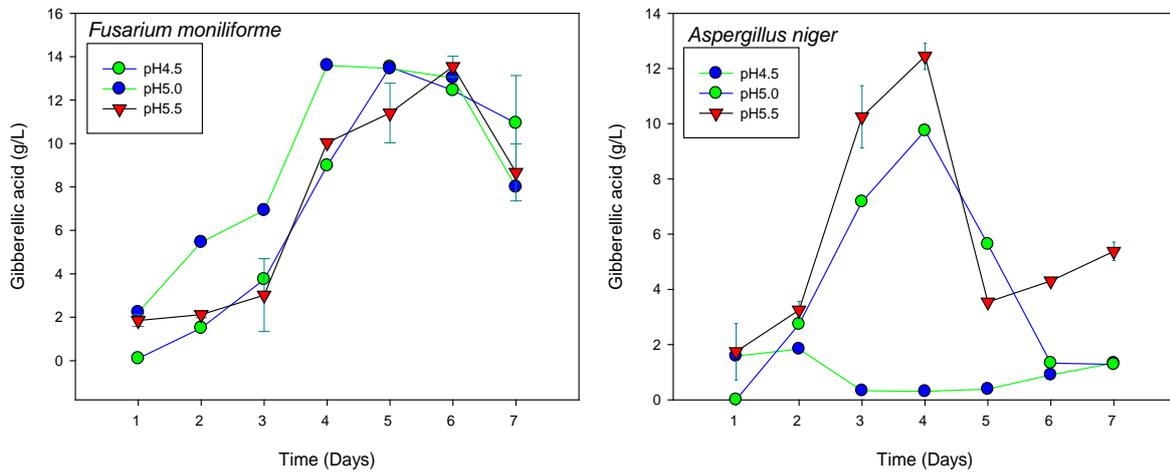


Fig. 2. Effect of varying pH on gibberellic acid yield using *F. moniliforme* and *A. niger* grown on banana peel as substrate

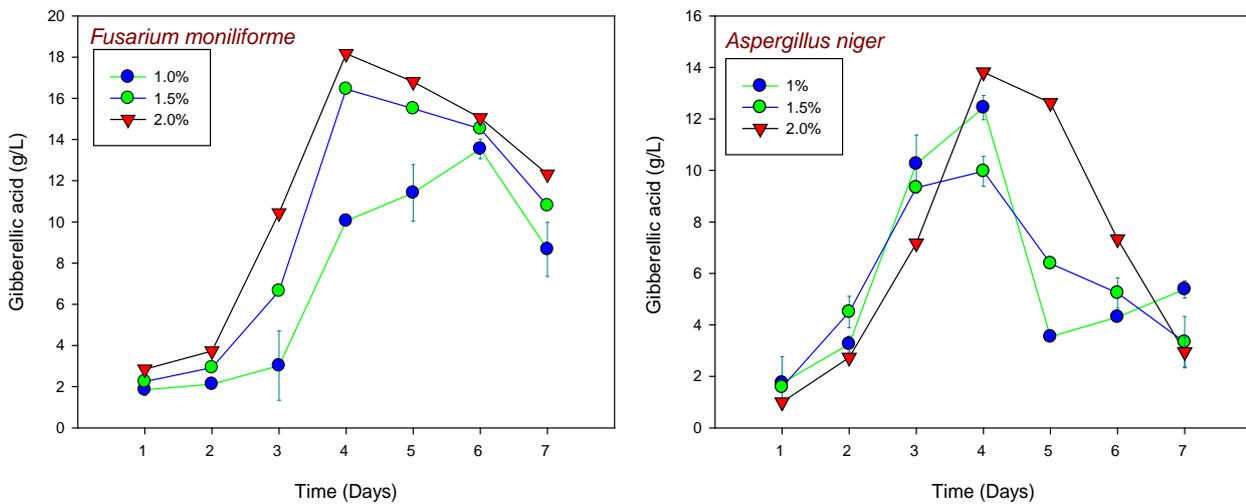


Fig. 3. Effect of varying inoculum size on gibberellic acid yield using *F. moniliforme* and *A. niger* grown on banana peel as substrate

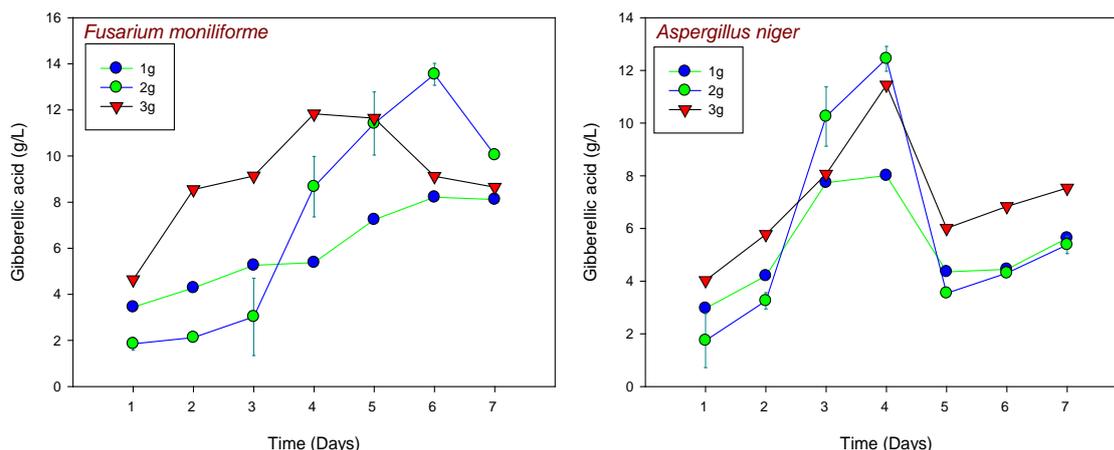


Fig. 4. Effect of varying substrate concentration on gibberellic acid yield using *F. moniliforme* and *A. niger* grown on banana peel as substrate

Table 2. Gibberellic acid production by *F. moniliforme* and *A. niger* grown on banana peel supplemented with different concentrations of copper sulphate

Time (Days)	Gibberellic acid (g/L)							
	<i>Fusarium moniliforme</i>				<i>Aspergillus niger</i>			
	0.02% CuSO ₄	0.05% CuSO ₄	0.08% CuSO ₄	Control	0.02% CuSO ₄	0.05% CuSO ₄	0.08% CuSO ₄	Control
1	1.49 ± 0.06 ^a	1.89 ± 0.27 ^a	0.78 ± 0.15 ^b	1.85 ± 0.16 ^a	1.65 ± 0.07 ^a	1.46 ± 0.16 ^a	1.02 ± 0.05 ^b	1.74 ± 0.59 ^a
2	2.05 ± 0.20 ^a	1.91 ± 0.06 ^a	2.65 ± 0.17 ^a	2.12 ± 0.08 ^a	3.14 ± 0.16 ^a	1.79 ± 0.95 ^b	2.75 ± 0.05 ^a	3.25 ± 0.18 ^a
3	2.59 ± 0.06 ^b	3.44 ± 0.13 ^a	2.25 ± 1.08 ^b	3.02 ± 0.97 ^a	2.77 ± 0.06 ^b	2.65 ± 0.05 ^b	2.72 ± 0.04 ^b	10.25 ± 0.65 ^a
4	3.52 ± 0.06 ^c	3.68 ± 0.09 ^b	4.55 ± 0.05 ^a	10.04 ± 0.02 ^a	9.14 ± 0.05 ^b	9.27 ± 0.16 ^b	9.22 ± 0.10 ^b	12.44 ± 0.27 ^a
5	7.92 ± 0.10 ^b	4.29 ± 0.08 ^c	10.26 ± 0.36 ^a	11.41 ± 1.37 ^a	3.36 ± 0.15 ^a	3.34 ± 0.08 ^a	3.01 ± 0.13 ^a	3.54 ± 0.03 ^a
6	10.05 ± 0.08 ^b	9.45 ± 1.11 ^b	10.78 ± 0.06 ^b	13.55 ± 0.27 ^a	4.05 ± 0.05 ^a	4.42 ± 0.10 ^a	4.52 ± 0.21 ^a	4.31 ± 0.01 ^a
7	3.71 ± 0.13 ^c	9.33 ± 0.06 ^a	4.83 ± 0.17 ^b	8.67 ± 1.33 ^a	3.81 ± 0.05 ^b	4.18 ± 0.04 ^b	3.67 ± 0.06 ^b	5.38 ± 0.19 ^a

Values represented are means ±SD of amount of Gibberellic acid. Means with the same superscript across a column are not statistically different from each other

Table 3. Production of gibberellic acid by submerged fermentation of banana peel using *F. moniliforme* and *A. niger* under optimized conditions

Time (Days)	Gibberellic acid (g/L)			
	<i>Fusarium moniliforme</i>		<i>Aspergillus niger</i>	
	Banana peel	CMC (Control)	Banana peel	CMC (Control)
1	1.86 ± 0.06 ^a	0.91 ± 0.03 ^b	1.45 ± 0.03 ^a	1.31 ± 0.03 ^a
2	8.64 ± 0.02 ^a	2.75 ± 0.03 ^b	2.24 ± 0.08 ^a	1.81 ± 0.03 ^b
3	9.45 ± 0.03 ^a	5.25 ± 0.02 ^b	4.79 ± 0.05 ^a	2.21 ± 0.03 ^b
4	9.28 ± 0.01 ^a	5.56 ± 0.06 ^b	9.35 ± 0.65 ^a	2.39 ± 0.03 ^b
5	12.11 ± 0.06 ^a	6.84 ± 0.06 ^b	13.50 ± 0.07 ^a	3.45 ± 0.04 ^b
6	17.48 ± 0.10 ^a	7.03 ± 0.04 ^b	6.56 ± 0.04 ^a	4.28 ± 0.04 ^b
7	12.85 ± 0.04 ^a	3.80 ± 0.09 ^b	6.43 ± 0.05 ^a	1.85 ± 0.03 ^b

Values represented are means ±SEM of gibberellic acid produced. Means with the same superscript in a row are not statistically different from each other (*F. moniliforme* and *A. niger* were compared separately)

The results of the infra-red spectroscopy of the recovered GA, the major characteristic peaks obtained by FT-IR in (A) curve, standard gibberellic acid, were at 1,169 cm⁻¹ (C-O group); 1,656 cm⁻¹ (C=C), 1745 cm⁻¹ (C=O); and 3,449 cm⁻¹ (OH group), while they were obtained at 1,115 cm⁻¹ (C-O); 1,637 cm⁻¹ (C=C) and 3,455 cm⁻¹ (OH group) for extracted GA (Fig. 4).

The proximate composition of BP used in the current study was within the range reported by other researchers; 40-64 % carbohydrate, protein 0.09-10.44%, 17-35% crude fibre and 0.85-13.4% ash (Anhwange, 2008; Romelle *et al.*,

2016; Morais *et al.*, 2017). In addition to these, BP has been shown to be rich in essential amino acids, dietary fibre, polyunsaturated fatty acids, iron and potassium (Ibitoye, 2005; Juarez-Garcia *et al.*, 2006). All these are relevant for a proper growth of the fermenting organisms and subsequent production of GA. Gibberellin production is reported to commence during the fermentation when nitrogen is exhausted in the medium and continues in the presence of adequate residual carbon in the substrate (Escamilla *et al.*, 2000). Therefore, a good substrate should provide adequate nutrients for an initial mycelial growth in a nitrogen-limited

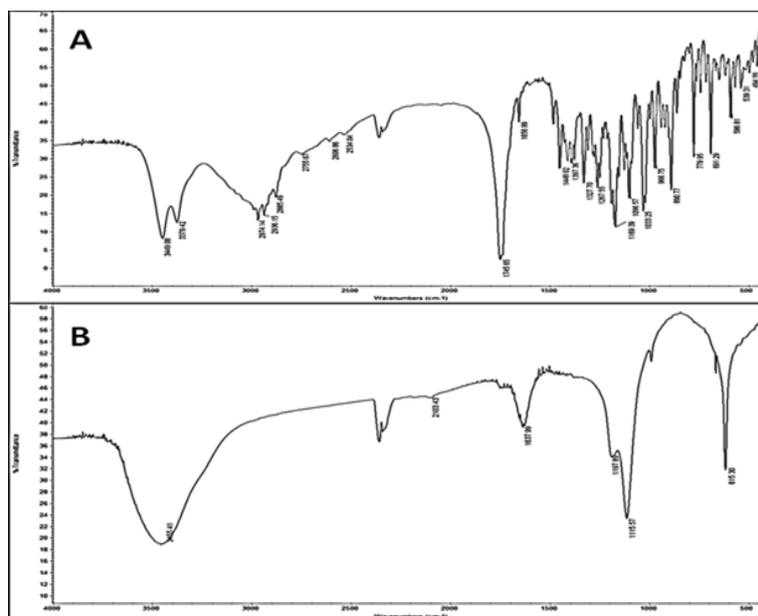


Fig. 4. FT-IR spectra of standard gibberellic acid (A) and extracted gibberellic acid (B)

but balanced medium (Rodrigues *et al.*, 2009). The amount of carbohydrates (42.62%) and proteins (9.54%) recorded in the BP substrate fits the high C/N ratio recommended in substrates for GA production (Kumar and Lonsane, 1990).

In the pre-optimization fermentation, *F. moniliforme* yielded significantly higher GA amounts than *A. niger* (Table 1). This GA yield exceeded most amounts reported by other researchers. Yields of 0.7 g/L using *G. fujikuroi* (Lale *et al.*, 2006); 1.82 g/L by *F. moniliforme* (Kobomoje *et al.*, 2013); 2.8g/L by *F. moniliforme* (Pastrana *et al.*, 1993); 11.3 g/L by *F. moniliforme* (Bilkay *et al.*, 2010) and 460.06 mg/L by *G. fujikuroi* were reported by Cuali-Alvarez *et al.* (2011). However, higher yields of 15 g/L and 32.8 g/L have been reported by Rangaswamy (2012) and Omojasola and Benu (2016) on *Jatropha* seedcake using *F. moniliforme* and *A. terreus* respectively. Rangaswamy (2012) reported 105 mg/g yield using solid state fermentation. Differences in yield may be attributed to differences in the substrates and organisms used in the fermentations. The fungi used in this study are reported to be highly cellulolytic fungi (Dashtban *et al.*, 2009) and the yield of GA obtained can be attributed to their ability to efficiently utilize cellulosic components of the substrate.

Regarding the fermentation time, maximum production was observed on day 6 and day 4 by *F. moniliforme* and *A. niger* respectively after which the yield began to decrease. It was observed that GA production initiated on the first day of fermentation, which is contrary to reports that GA commences about 46 h after the initiation of fermentation following nitrogen depletion in the medium (Escamilla *et al.*, 2000; Rodrigues *et al.*, 2009; Rios-Irube *et al.*, 2011). However, Lale and Gadre (2010) also reported GA production at 20 h by a mutant of *G. fujikuroi*. Maximum GA yields are reported to be between days 4 and 8 of fermentation for *F. moniliforme* (Kumar and Lonsane, 1990; Meleigy and Khalaf, 2009; Rangaswamy, 2012; Omojasola and Benu, 2016) and day 6 and 12 for *A. niger* (Bilkay *et al.*, 2010).

The initial pH of batch-fed fermentations is one of the most important factors in GA production. It not only affects the growth of the fungi, but also influences their physiological activities. Escamilla *et al.* (2000) reported that high pH neutralizes acid production e.g. Le Chatelier's principle; therefore, the highest GA production is recorded at low C:N ratios and large pH. It was observed that GA yield was maximized at pH 5.0 by *F. moniliforme*, although no significant differences were observed between the yields obtained at pH 5.0 and 5.5 on the days 6 and 7 of fermentation and pH 5.5 by *A. niger*. This corresponds with the studies of Kahlon and Mahlotra (1986), Shukla *et al.* (2005), Bilkay *et al.* (2010) and Kobomoje *et al.* (2013) who reported maximum GA yields between pH 5.0 and 5.5.

Maximum yield of GA was observed when 2% inoculum size was used. Although there was no significant difference between the peak yields obtained with 1.5% and 2% inocula, the latter quantitatively posted the higher yield. The amount of inoculum is important because low amounts may give inadequate biomass and lead to a reduction in the GA yield, while excessive inoculum may form superfluous biomass causing a significant exhaustion of the nutrients that should otherwise have been utilized for the production of GA (Karthikeyan and Sivakumar, 2010). This is similar to the findings of Omojasola and Benu (2016), although Kobomoje *et al.* (2013) reported a lower optimum inoculum size of 1.5%

Substrate concentration of 2 g gave the highest GA yield by both test fungi. However, there were statistical similarities in the yields obtained using 2 g and 3 g of BP substrate. The use of a variety of substrates and their GA yields has been cited in literature. Yields of 25 g/L and 32.8 g/L at 1.5 g and 3.5 g *Jatropha* seed cake residue was reported by Omojasola and Benu (2016), while Kobomoje *et al.* (2013) 1.82 mg/ml at 10% substrate concentration using shea nut shell.

The supplementation of the medium with different concentrations CuSO₄ showed little or no significant effect at all concentrations used for the BP substrate (Table 2).

This suggested that addition of CuSO_4 to fermentation media, at least at the concentrations used has no influence on the yield of gibberellic acid. This result is a dissimilarity with the study of Chinedu *et al.* (2011) who used the supplement for improved production of cellulase by *Aspergillus niger*, *Penicillium chrysogenum* and *Trichoderma harzianum*.

The results of the infra-red spectroscopy of the extracted GA using FT-IR showed it contained three out of four of the main characteristic bands present on the standard GA sample (Fig. 5). This is similar to the range of absorption bands reported by Sleem (2013) with the exception of the carbon hydrogen group. The absence of the carbon hydrogen group could be due to its metabolism by the fermenting fungi.

The yields obtained by *F. moniliforme* and *A. niger* in the optimized fermentation were 17.48 g/L (29.1% increase) and 13.50 g/L (8.52% increase) respectively.

Conclusions

The current results obtained have shown the potential of BP as a good substrate for the production of gibberellic acid. The yields obtained in the present study are among the highest that are available in literature and the data have supported the effective utilization of BP for the fermentative production of gibberellic acid. The hereby study, therefore, provided a dual importance of utilizing an agro-industrial waste for the bioproduction of GA and reducing of environmental pollution.

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