Fjrs.futa.edu.ng
FUTA Journal of Research in Sciences
ISSN: 2315 – 8239 (Print); E-ISSN: 2489 - 0413

FUTA Journal of Research in Sciences, Vol. 12 (2) 2016: 242-251

# FERMENTATIVE PRODUCTION OF GIBBERELLIC ACID FROM *JATROPHA CURCAS* SEED CAKE USING *ASPERGILLUS NIGER* AND *ASPERGILLUS TERREUS*

**P.F. Omojasola* and O. E. Benu**

Department of Microbiology, Faculty of Life Sciences, University of Ilorin, P.M.B. 1515, Ilorin, Kwara State. Nigeria
*Corresponding author's email: folakejasola@yahoo.co.uk; jasola@unilorin.edu.ng

## ABSTRACT
Agro-industrial residues consist of various wastes from the agriculture and the food industry, which account for several thousand tonnes of waste per year. These wastes pollute the environment and represent a loss of biomass which can otherwise be processed to other value-added products through the activities of microorganisms. This study aims to ferment the seed cake of *Jatropha curcas* an agro-based waste, for the production of gibbererllic acid using *Aspergillus niger* (ATCC 16404) and *Aspergillus terreus* (ATCC 20542) in submerged fermentation. The seeds of *Jatropha* were cracked, ground and defatted. The ground seeds were pretreated with 0.5% tetraoxosulphate VI acid (w/v), and fermented separately in a modified Czapek Dox broth using the test organisms. Carboxyl methylcellulose (CMC) was used as control. The fermentation conditions were: pH 5.3; substrate concentration (1.5g); inocula size 2 mL; ($1.2 \times 10^7$ spores/mL *A. niger*) ($5.83 \times 10^6$ spores/mL *A. terreus*); temperature 25°C; fermentation period of 7 days. Gibberellic acid yield of 13.8±0.97 g/L and 12.2±0.97 g/L was produced by *A. niger* and *A. terreus* respectively. The yield from the CMC substrate was 5.60±0.49 and 2.0±0.32 g/L from *A. niger* and *A. terreus* respectively. The fermentation parameters were then varied in order to optimize the yield of the acid. The highest yield of gibberellic acid was 31.0±0.23 g/L and 32.8±0.25 g/L at pH 5, substrate concentration 1.5g, inocula size 2 mL for 8 days; and pH 7, substrate concentration 3.5g and inocula size 2 mL for 8 days with *A. niger* and *A. terreus* respectively, representing a 55% and 63% yield increase by *A. niger* and *A. terreus* respectively. These results support the potential use of *Jatropha* seed cake for gibberellic acid production.
**Keywords**: Gibberellic acid, *Aspergillus niger*, *Aspergillus terreus*, *Jatropha curcas*, submerged fermentation

## INTRODUCTION
Gibberellic acid (also called Gibberellin A3, GA, and $GA_3$) is a hormone found in plants with chemical formula $C_{19}H_{22}O_6$ (Rodrigues *et al.*, 2012). When purified, it is a white to pale-yellow solid. Gibberellic acid is a very potent hormone whose natural occurrence in plants controls their development (Riley, 1987). GA was first identified in Japan in 1935, as a metabolic byproduct of the plant pathogen *Gibberella fujikuroi* (thus the name), which afflicts rice plants; *fujikuroi*-infected plants develop *bakanae* ("foolish seedling"), that causes them to grow so much taller than normal that they die from no longer being sturdy enough to support their own weight (Riley, 1987). Gibberellins have a number of effects on plant development. It stimulates rapid stem and root growth, induces mitotic division in the leaves of some plants, eliminates dormancy, flowering, sex expression, leaf and fruit senescence and increases seed germination rate (Kahlon and Malhotra, 1986;

Rangaswamy, 2012). Gibberellic acid is sometimes used in laboratory and greenhouse settings to trigger germination in seeds that would otherwise remain dormant. GA has been shown to increase shoot length, mobilization efficiency, emergence index, speed and coefficient of germination (Jefferys, 1970; Utkarsha *et al.,* 2011). GA is a highly valued industrially important biochemical with various applications in agriculture. The price is around $25/g in the international market (Avinash *et al*., 2003). GA is presently produced largely by submerged fermentation techniques using *Fusarium moniliforme* or *Gibberella fujikuroi* (Santos *et al*., 2003).

*Jatropha* contains about 30-35% (w/w) oil leaving behind press cake (75% including about 5% losses of oil in extraction process in the mechanical expeller) with residual oil (Makkar *et al.,* 2008). The press cake is rich in organic matter, dark brown to black in color and contains carbohydrates, fibers, residual oil and is useful as organic fertilizer because of the high content of nitrogen. The cake is also found to contain large amounts of water and carbon content along with low contents of hydrogen and oxygen (Mohit *et al.,* 2011). *Jatropha* seed cake is one of the best carbon sources among various carbohydrates, because it is pure, inexpensive and available in a mass supply (Rao *et al*., 2008). The protein-rich seed cake however contains anti-metabolic and toxic principles including trypsin inhibitor, lecithin, saponin and phytate (Makkar *et al.,* 1997; Abdo and Juamat, 2009; Ncube *et al.,* 2012) which no treatment has been able to completely eliminate, hence the *Jatropha* seed cake is regarded as agro-waste (Martinez-Herrera *et al.,* 2006; Rakshit *et al.,* 2008).

The objectives of this study were to determine the suitability of *Jatropha* seed cake (JSC) as a cellulosic substrate for the production of gibberellic acid; to determine the ability of *Aspergillus niger* and *Aspergillus terreus* to utilize JSC as substrate for gibberellic acid production and optimal conditions for the production under laboratory conditions.

## MATERIALS AND METHODS

### Collection of sample and test organisms

*Aspergillus niger* ATCC 16404 and *Aspergillus terreus* ATCC 20542 were obtained from the Federal Institute of Industrial Research, Oshodi (FIIRO), Lagos, Nigeria. These were cultured and maintained on Potato Dextrose Agar slants at $4^{o}$C until use. The *Jatropha* seeds were collected from the Department of Crop Science, Faculty of Agriculture, University of Ilorin, Ilorin, Kwara State, Nigeria.

### Proximate analysis of *Jatropha* seed cake

The proximate analysis of the JSC was carried out. The parameters investigated were moisture content, ash, crude protein, total carbohydrate and crude fibre (AOAC, 2000), crude lipids (Parkouda *et al.,* 2008).

### Spore suspension

Fungal spore inoculum was produced by washing spores of a 7-day old culture of each test fungus in sterile distilled water and adjusting appropriately to $1.2 \times 10^{7}$ spores $mL^{-1}$ for *A. niger* and $5.83 \times 10^{6}$ spores $mL^{-1}$ for *A. terreus*. The size of the inoculum was determined by counting using the improved Neubauer haemacytometer.

### Substrate pretreatment

The seeds were cracked and ground using a blender, (Nakai, Japan), this was defatted by soaking in petroleum ether for 24 hours and sieved. The ground seeds were then dried and pretreated with equal volume of 0.5% $H_2SO_4$ (w/v), autoclaved and dried again (Mohit, 2011). The resulting cake was then ground again before use.

**Fermentation media**

The media used for gibberellic acid fermentation was a modified Czapek Dox broth using the method described by Rangaswamy (2012) with 50% replacement of glucose with *Jatropha* seed cake. Composition was 1.5g *Jatropha* seed cake, 1.5g glucose, 0.3g $NaNO_3$, 0.1g $K_2HPO_4$, 0.05g $MgSO_4.7H_2O$, 0.05g and KCl, 0.001 $FeSO_4$.

**Submerged fermentation**

The test organisms were drawn separately from the spore suspension and each inoculated into 100 mL of sterile fermenting medium. Gibberellic acid production was monitored every 24 h. The fermentation was incubated at $30^oC$ on a rotary shaker (Gallenkamp, England) at 150 rpm for 8 days. The final pH was adjusted using 0.2M NaOH or 0.1M HCl. Gibberellic acid was estimated in the supernatant spectrophotometrically (Jenway 6105 UV/VIS) using the method described by Berrios *et al.* (2004) at 254 nm. The culture media was filtered, acidified to pH 2.5 with HCl and extracted using liquid-liquid (ethylacetate/$NaHCO_3$) extraction (Bilkay *et al.,* 2010). Gibberellic acid in ethylacetate phase was measured by UV spectrophotometer. The amount of gibberellic acid was calculated from the standard curve (Berrios *et al.,* 2004).

**Optimization of gibberellic acid production**

The optimization experiments were:

i. Effect of varying pH: pH of medium was varied between 3.0 – 7.0
ii. Effect of varying substrate concentration: substrate concentration was varied between 0.5 - 4.5g
iii. Effect of varying inoculum size: inoculum size was varied between 2.0 – 6.0 mL. ( *A. niger* inoculum = 1.2 x $10^7$ spores $mL^{-1}$; *A. terreus* inoculum = 5.83 x $10^6$ spores $mL^{-1}$)
iv. Effect of varying fermentation time: The fermentation period was increased from 8 to 15 days.

**RESULTS**

The data from this study confirmed that *Jatropha curcas* seed cake is a suitable substrate for gibberellic acid production by fungal fermentation using *Aspergillus niger* (ATCC 16404) and *Aspergillus terreus* (ATCC 20542). JSC was a better substrate than CMC used as control which gave lower gibberellic acid yields (Table 1). The result of the proximate composition of the *Jatropha curcas* seed cake analyzed were: lipids 32.13±0.05%; protein 29.34±0.02%; carbohydrate 16.23±0.02%; moisture 6.15±0.13%; ash content 5.75±0.03%, and crude fibre 10.42±0.01%. These serve as nutrients, raw materials and carbon source that can be converted into valuable sugar by microorganisms for the production of gibberellic acid.

**Table 1: Production of gibberellic acid by submerged fermentation from *Jatropha* seed cake using *Aspergillus niger* and *Aspergillus terreus***

| Fermentation time (Days) | Gibberellic Acid (g $L^{-1}$) | | | |
|---|---|---|---|---|
| | *Aspergillus niger* | | *Aspergillus terreus* | |
| | JSC | (CMC) Control | JSC | (CMC) Control |
| 1 | $7.6\pm0.42^{a}$ | $3.0\pm0.14^{a}$ | $7.2\pm0.42^{b}$ | $3.5\pm0.42^{b}$ |
| 2 | $8.4\pm0.55^{b}$ | $4.05\pm0.34^{b}$ | $8.8\pm0.55^{ab}$ | $4.0\pm0.52^{a}$ |
| 3 | $9.8\pm0.60^{b}$ | $4.10\pm0.35^{b}$ | $8.9\pm0.59^{a}$ | $3.2\pm0.34^{b}$ |
| 4 | $8.4\pm0.30^{c}$ | $4.0\pm0.16^{c}$ | $6.2\pm0.30^{a}$ | $3.2\pm0.35^{ab}$ |
| 5 | $6.8\pm0.08^{a}$ | $5.0\pm0.71^{a}$ | $3.6\pm0.08$ | $3.5\pm0.37^{a}$ |
| 6 | $12.0\pm0.08^{b}$ | $4.5\pm0.35^{b}$ | $2.4\pm0.08$ | $2.2\pm0.30^{a}$ |
| 7 | $13.8\pm0.98^{c}$ | $5.6\pm0.49^{c}$ | $12.2\pm0.97^{c}$ | $2.0\pm0.32^{a}$ |
| 8 | $11.6\pm0.18^{c}$ | $5.8\pm0.67^{c}$ | $2.4\pm0.18^{a}$ | $4.0\pm0.49^{c}$ |

(Substrate concentration: 1.5g, pH:5.3, inocula size: 2mL, temp 29±1°C). Values represented are means SD ± of amount of Gibberellic acid. All groups are compared at $p<0.05$. Values having different superscripts are statistically different.

Results from the pre-optimization fermentation showed that GA production peaked on Day 7 of fermentation with a yield of 13.8 g/L and 12.2 g/L by *A. niger* and *A. terreus* respectively (Table 1). Thereafter, the GA yield dropped. The fermentation was optimized by varying the conditions of fermentation. The effect of varying fermentation time was recorded as increase in GA production (Fig. 1). The highest GA yields of 24 g/L by *A. niger* and 20 g/L by *A. terreus* were recorded on Days 6 and 11 respectively (Fig. 1). Substrate concentration was varied between 0.5-4.5g; with the highest GA yields of 24 g/L at 1.5 g from *A. niger* and 23 g/L at 3.5 g substrate concentration from *A. terreus* (Fig. 2).

The results of the pH optimization showed that the highest GA yield by *A. niger* was 19.8 g/L on Day 6 at pH 5.0 and 18 g/L at pH 7.0 on Day 8 by *A. terreus* (Fig. 3). The results of varying inoculum size indicate that 2 mL gave highest GA yield of 24 g/L for *A. niger* and 4 mL for *A. terreus* yielding 20.4 g/L (Fig. 4).

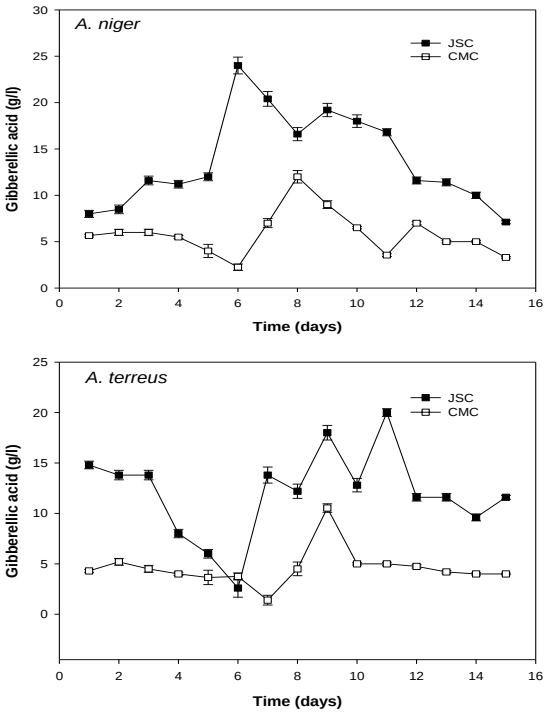


**Fig. 1: Effect of varying fermentation time on gibberellic acid production by *A. niger* and *A. terreus* using *Jatropha* seed cake**

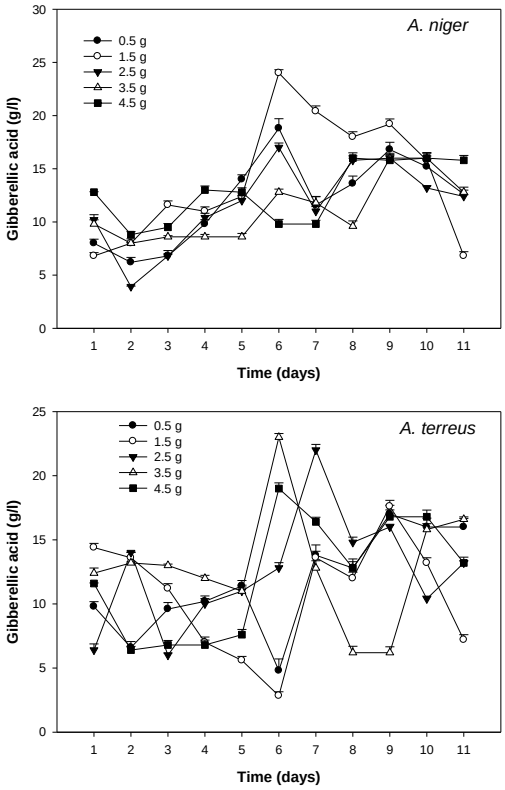


**Fig. 2: Effect of varying substrate concentration on gibberellic acid production by *A. niger* and *A. terreus* using *Jatropha* seed cake**

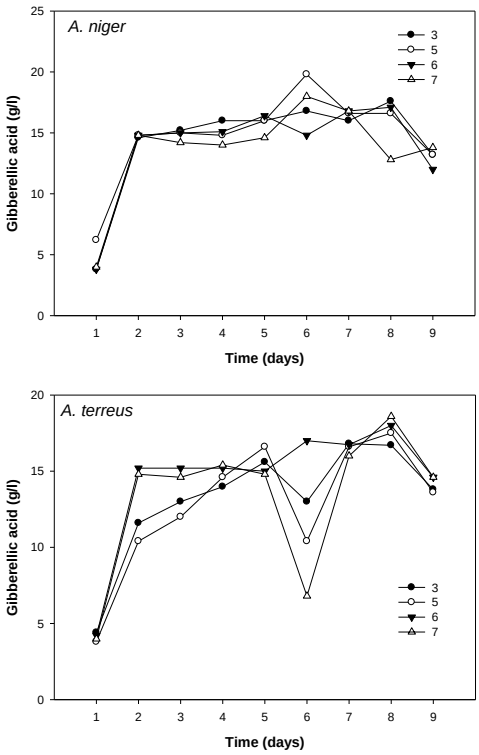


**Fig. 3: Effect of varying pH on gibberellic acid production by *A. niger* and *A. terreus* using *Jatropha* seed cake**

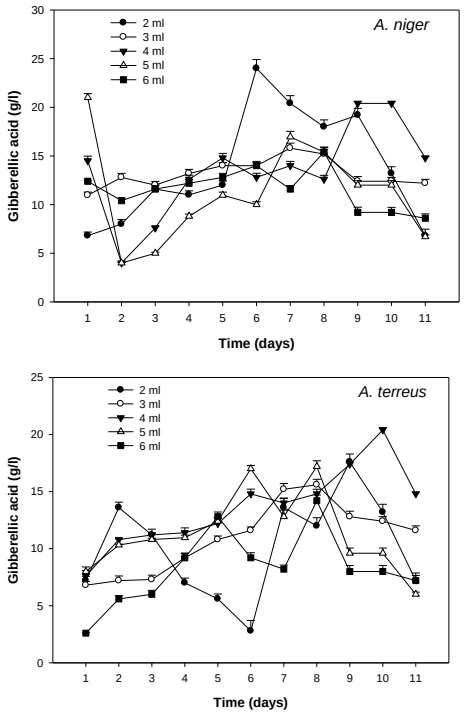


**Fig. 4: Effect of varying inoculum size on gibberellic acid production by *A. niger* and *A. terreus* using *Jatropha* seed cake**

All the conditions that yielded the highest amounts of GA in the optimization fermentations were combined in a single fermentation. This optimized fermentation produced a GA yield of 31.0 g/L on Day 8 at a pH of 5, substrate concentration of 1.5g/100 mL and inocula size of 2 mL with *A.niger*, while with *A. terreus* a yield of 32.8 g/L on Days 7 and 8 at a pH of 7, substrate concentration of 1.5 g/100mL and an inocula size of 2 mL (Table 2).

**Table 2: Optimized production of gibberellic acid from *Jatropha curcas* seed cake using *Aspergillus niger* and *Aspergillus terreus***

| Fermentation time (Days) | Gibberellic Acid (g/L) | | | |
|---|---|---|---|---|
| | *Aspergillus niger* | | *Aspergillus terreus* | |
| | JSC | (CMC) Control | JSC | (CMC) Control |
| 1 | $17.2 \pm 0.16^{a}$ | $6.2 \pm 0.02^{a}$ | $21.2 \pm 0.1^{a}$ | $10.0 \pm 0.1^{a}$ |
| 2 | $18.0 \pm 0.20^{ab}$ | $8.0 \pm 0.05^{b}$ | $20.6 \pm 0.002^{a}$ | $8.0 \pm 0.22^{b}$ |
| 3 | $14.8 \pm 0.16$ | $5.5 \pm 0.12^{a}$ | $20.8 \pm 0.027^{a}$ | $9.0 \pm 0.41^{b}$ |
| 4 | $14.8 \pm 0.08$ | $5.5 \pm 0.97^{a}$ | $20.8 \pm 0.025^{a}$ | $9.5 \pm 0.35^{a}$ |
| 5 | $20.4 \pm 0.08^{b}$ | $7.2 \pm 0.15^{ab}$ | $20.8 \pm 0.01^{a}$ | $9.75 \pm 0.37^{b}$ |
| 6 | $25.0 \pm 0.03^{bc}$ | $9.0 \pm 0.26^{a}$ | $21.2 \pm 0.01^{a}$ | $10.0 \pm 0.30^{a}$ |
| 7 | $21.0 \pm 0.39^{a}$ | $8.0 \pm 0.03^{a}$ | $32.8 \pm 0.008^{b}$ | $16.2 \pm 0.32^{a}$ |
| 8 | $31.0 \pm 0.23^{c}$ | $15.5 \pm 0.6^{ab}$ | $32.8 \pm 0.25^{a}$ | $16.4 \pm 0.49^{c}$ |
| 9 | $18.0 \pm 0.02^{a}$ | $8.0 \pm 0.14^{a}$ | $29.0 \pm 0.04^{ab}$ | $15.0 \pm 0.41^{a}$ |
| 10 | $17.0 \pm 0.03^{a}$ | $6.0 \pm 0.30^{b}$ | $17.2 \pm 0.05^{a}$ | $12.0 \pm 0.43^{a}$ |

(*A.niger:* Substrate concentration: 1.5g, pH:5, inocula size: 2mL; *A.terreus:* pH:6, substrate concentration:1.5g,inocula size: 2ml). Values represented are means SD ± of amount of gibberellic acid produced. All groups are compared to each other at $p<0.05$.Values having different superscripts are statistically different.

## DISCUSSION

*J. curcas* has been reported to contain between 28.87 – 37.82% crude protein, 11.92 – 13.18% carbohydrate and 29.95 – 37.85% (Belewu and Sam, 2010; Inekwe *et al.,* 2012; Phengnuam and Suntornsuk, 2013) Lipids are known to be superior energy sources and plant oils have been proven to contain about 2.5 times the energy content of glucose per weight basis: 8880 kcal/kg oil versus 3722 kcal/kg glucose (Omojasola and Sanu, 2013/14). *Aspergillus* are known to be cellulolytic which will assist in the degradation of the JSC (Dashtban *et al.,* 2009). The presence of a high C/N ratio is recommended for GA production (Rodrigues *et al.,* 2009) and this was made possible by the presence of proteins and carbohydrates in the JSC substrate.

Results from the pre-optimization fermentation recorded yields of 13.8 g/L and 12.2 g/L by *A. niger* and *A. terreus* respectively on Day 7 of fermentation (Table 1). This exceeded yields of 0.13 g/L on Day 9 using the shell of *Vitellaria paradoxa;* 238.7 mg/L on Day 6 on Czapek Dox broth by *Fusarium moniliforme* reported by Kobomoje *et al.* (2013) and Seyis *et al.* (2010) respectively. Rangaswamy (2013) recorded GA yields of 5.8 g/L by *A. niger* on Day 8 also using Czapek Dox broth. Generally, GA levels reported in literature as reviewed by Kumar and Lonsane, (1989) are significantly lower than those reported in this study. Yields of 0.7 g/L by *G. fujikuroi* (Lale *et al.*,2006); 0.12 g/L from dairy waste (Duran-Paramo *et al*., 2004); and 2.862 g/L using immobilized *G. fujikuroi* mycelium in fluidized bioreactors (Escamilla *et al*,.2000)

The results of the optimization experiments showed the highest GA yields of 24 g/L by *A. niger* and 20 g/L by *A. terreus* were recorded on Days 6 and 11 respectively (Fig. 1). This delay in the production of GA was also size of inoculum are important factors that affect the course of fungal fermentation. observed in the optimized experiments where fermentation time was varied (Fig. 1). Gibberellic acid is a secondary metabolite of fungi that is secreted by the fermenting organism near the end of the growth phase usually at the stationary phase (Bilkay *et al.,* 2010). The production of GA starts when nitrogen has been depleted and continues when sufficient carbon concentration is available (Escamilla *et al.,* 2000; Rodrigues *et al.,* 2009).

Substrate concentration was varied between 0.5-4.5g; with the highest GA yields at 1.5 g and 3.5 g from *A. niger* and *A. terreus* respectively (Fig. 2). Kobomoje *et al.* (2013) reported highest GA yields at 1.60 g/L substrate concentration. Physiological factors considerably affect GA production in submerged fermentation (Kahlon and Malhotra, 1986; Karakoc and Aksoz, 2006).

The effect of pH on the production of gibberellic acid by the test organisms was investigated. It was noted that the initial pH did not greatly affect GA production (Fig 3). The highest yield by *A. niger* was 19.8 g/L on Day 6 at pH 5.0 and 18 g/L at pH 7.0 on Day 8 by *A. terreus* (Fig. 3). Similar profile was reported for GA production by *Fusarium moniliforme* where a maximum yield of 6.5 g/L at pH 7 on Day 8 (Rangaswamy, 2012); maximum yield of 0.3 g/L by *Pseudomonas* also at pH 7 (Karakoc and Aksoz, 2006); and maximum yield of 1.70 g/L by *F. moniliforme* at pH 5.5 (Kobomoje *et a*l. 2013). There are reports that GA production decreases when the pH was outside the 3.0-5.5 in a stirred culture (Borrow *et al.,* 1964; Rangaswamy, 2012). However, the results of this study do not support this.

Inoculum size that gave the highest GA yield was 2 mL (24 g/L) for *A. niger* and 4 mL for *A. terreus* (Fig. 4). Fungal type and

Several fungi are known to be cellulolytic and various species that have been used in

the fermentation of cellulosic biomass for GA production include *F. moniliforme* (Rangaswamy, 2012; Kobomoje *et al.,* 2013) *Gibberella fujikuroi* (O' Donnell *et al.,* 1998; Rodrigues *et al.,* 2009). It was also observed that further increase in inoculum size gave lower GA yields, possibly due to competition for nutrients and overcrowding of the fermenting organism.

The results of the optimized fermentation gave a yield of 31.0 g/L by *A. niger* and 32.8 g/L on Days 7 and 8 by *A. terreus* (Table 2). Rangaswamy (2012) reported 15 g/L of the acid by optimization on the physiological parameters in submerged fermentation with modified Czapek Dox medium which was reported as being 3-fold higher than highest yield reported in literature using submerged fermentation. This yield is double using the same mode (Table 2).

## CONCLUSION

The focus of this study was both to explore the use of JSC as suitable substrate for GA production and also optimization for increased GA yield using *A. niger* and *A. terreus.* The results obtained indicate that *Jatropha curcas* seed cake (JSC) is a suitable low cost fermentation substrate for the production of gibberellic acids. The yield from the substrate was compared with that from CMC (control) and it was observed that higher yields resulted from both organisms independently with JSC as substrate. Both fungi gave good yield; however, *A. terreus* gave a higher yield than *A. niger* resulting in a yield of 32.8 g/L while *A. niger* gave 31.0 g/L.

In conclusion, the data obtained in this study indicates a good yield of the acid depends on the presence of appropriate physical conditions and nutritional requirements during fermentation process using JSC as a substrate. Thus, it can be concluded that experimental strain of *A. niger* ATCC 16404 and *A. terreus* ATCC 20542 could be employed for producing the acid on a large scale as shown in the optimization process. As the use of JSC as a bio-fuel source is on the increase, the waste (seed cake) produced will also increase; using it for the production of these acids will help prevent environmental pollution and at the same time provide cheap substrate for the production process.

## REFERENCES

**Abdo, A.W. and Juamat, S.** (2009). Phorbol esters as toxic constituents of *Jatropha curcas* seed oil. European Journal of Scientific Research 31: 429-436.

**AOAC** (2000). Association of Official and Analytical Chemists. Official Methods of Analysis.17th Ed. Washington D.C.

**Avinash, C.S., Shahid, A., Agarwal, D.K., Sarbhoy, A.K.** (2003). Screening of potential gibberellin producing *Fusarium* strains for the hybrid rice production. Journal of Food Agriculture and Environment 1:250-253.

**Belewu, M.A. and Sam, R**. (2010). Solid state fermentation of *Jatropha curcas* kernel cake: Proximate composition and antinutritional components. Journal of Yeast and Fungal Research 1(3):44-46.

**Berrios, J., Illanes, A., Aroca, G**. (2004), Spectrophotometric method for determining Gibberellic acid in fermentation broths. Biotechnology Letters 26(1): 67-70.

**Bilkay, S. I., Karakoc, S. and Aksoz, N**. (2010). Indole-3-acetic acid and gibberellic acid production in *Aspergillus niger.* Turkish Journal of Biology 34:313-318 [doi:10.3906/biy-0812-15]

**Borrow, A., Brown, S., Jeffery E.G., Kessell, R.H.J., Lloyd, E.C., Lloyd P.B., Rothwell, A., Rothwell, B. and Swait, J.C.** (1964). The effect of varied temperature on the kinetics of

metabolism of *Gibberella fujikuroi* in a stirred culture. Canadian Journal of Microbiology 10: 445-466.

**Dashtban, M., Schraft, H. and Qin W. (2009).** Fungal bioconversion of lignocellulosic residues: Opportunities and perspectives. International Journal of Biological Sciences 5: 578-595

**Duran-Paramo, E., Molina – Jimenez, H., Brito-Arias, M.A., Robles-Martinez, F.** (2004) Gibberellic Acid Production by Free and Immobilized Cells in different culture systems. Applied Biochemistry and Biotechnology 113-116, 381-388.

**Escamilla, E.M., Dendooven, L., Magana, I.P., Parra, R., De La Torre, M**. (2000). Optimization of gibberellic acid production by immobilized *Gibberella fujikuroi* mycelium in fluidized bioreactors Journal of Biotechnology 76:147-155.

**Inekwe, U.V., Onyike, E., Odey, M.O., Agbaji, A.S., Joel, J.T. and Diafe, P**. (2012). Comparative proximate composition of *Jatropha curcas* seed from India, Kaduna and Edo. International Journal of Science and Technology 2(6):379-381

**Jefferys, E.G.** (1970). The gibberellins fermentation. Advances in Applied Biology. 13: 283-316.

**Kahlon, S.S. and Malhotra, S.** (1986). Production of gibberellic acid by fungal mycelium immobilized in sodium alginate. Enzyme and Microbial Technology 8: 613-616

**Karakoc, S. and Aksoz, N**. (2006). Some optimal cultural parameters for gibberellic acid biosynthesis by *Pseudomonas* sp. Turkish Journal of Biology 30:81-85

**Kobomoje, O.S., Mohammed, A.O. and Omojasola, P.F.** (2013). The production of substrate for production of xylanase and cellulase by *Aspergillus niger* gibberellic acid from shea nut shell (*Vitellaria paradoxa*) using *Fusarium moniliforme*. Asian Journal of Plant Science and Research 3(2):23-26.

**Kumar, P.K.R. and Lonsane, B.K.** (1989). Microbial production of gibberellins: state of the art. Advances in Applied Microbiology. 34**:** 29–138.

**Lale, G. Jogdand, V.V. and Gadre, R.V.** (2006). Morphological mutants of *Gibberrella fujikuroi* for enhanced production of gibberellic acid. Journal of Applied Microbiology 100:65-72.

**Makkar, H.P.S., Becker, K., Sporer, F., and Wink, M.** (1997). Studies on nutritive potential and toxic constituents of different provenances of *Jatropha curcas.* Journal of Agricultural and Food Chemistry 45:3152-3157.

**Makkar, H.P.S., Francis, G., and Becker, K.** (2008). Protein concentrate from *Jatropha curcas* screw pressed seed cake and toxic and anti-nutritional factors in protein concentrate. Journal of the Science of Food and Agriculture 88: 542-548.

**Martinez-Herrera, J., Siddhuraju, P., Francis, G., Davila-Ortiz, G., Becker, K.** (2006). Chemical compositiontoxic/antimetabolicconstituents and effects of different treatments on their levels, in four provenances of *Jatropha curcas* L. from Mexico. Food Chemistry 96: 80-89.

**Mohit, S. Mishra, Chandrashekhar, B., Tanushree C. and Kanwal, S**. (2011). Production of bio-ethanol from *Jatropha* oilseed cakes via dilute acid hydrolysis and fermentation by *Saccharomyces cerevisiae* International Journal of Biotechnology Applications. 3(1): 441-47.

**Ncube, T., Howard, R.L., Abotsi, E.K., Jansen van Rensburg, E.L and Ncube I.** (2012). *Jatropha curcas* seed cake as

FGSCA733 in solid state fermentation. Industrial Crops and Products 37: 118-123.

**O'Donnell, K., Cigelnik, E. and Nirenberg, H.I.** (1998). Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. Mycologia 90:465-493.

**Omojasola, P.F. and Sanu, F.T**. (2013) Microbiological Quality Assessment of Dried Yam Chips (*Dioscorea rotundata)* During Storage. Journal of Microbiology Biotechnology and Food Sciences 3(3): 208-213.

**Parkouda, C., Diwara, B. and Ouoba, L.I.V.** (2008). Technology and physicochemical characteristics bilkalga alkaline fermented seeds of *Hibiscus sabdariffa.* African Journal of Biotechnology 7(7):916-922.

**Phengnuam, T. and Sutornsuk, W.** (2013). Detoxification and anti-nutrients reduction of *Jatropha curcas* seed cake by *Bacillus* fermentation. Journal of Bioscience and Bioengineering 115(2):168-172 [doi:10.1016/j.biosc.2012.08.017]

**Rakshit, K.D., Darukeshwara, J., Rathina,R.K., Narasimhammurthy, K., Saibaba, P. and Bhagya, S.** (2008). Toxicity studies on of etoxified *Jatropha* (meal) *J. curcas* in rats. Food Chemistry and Toxicology 46:3621-3625.

**Rangaswamy, V.** (2012). Improved production of gibberellic acid by *Fusarium moniliforme.* Journal of Microbiology Research. 2(3): 51-55 [doi: 10.5923/j.microbiology.20120203.02].

**Rao, G.R., Korwar, G.R., Shankar, A.R. and Ramakrishna, Y.S.** (2008). Genetic associations, variability and diversity in seed characters, growth reproductive phenology and yield in *Jatropha curcas* L. accessions. Trees 22(5):697-709. Doi 10.1007/s00468-008-0229-4

**Reig, C., Farina, V., Volpe, G., Mesejo, C., Martinez-Fuentes, A., Barone, F., Calabrese, F., and Agusti, M.** (2011). Gibberellic Acid and Flower bud Development in Loquat. Scientia Horticulturae 129(1): 27-33.

**Riley, J M.** (1987). Gibberellic Acid for Fruit Set and Seed Germination. CRFG Journal 19:10-12

**Rodrigues, C., Vandenberghe, L.P., Teodoro, J., Oss, J.F., Pandey, A. and Soccol, C.R.** (2009). A new alternative to produce gibberellic acid by solid state fermentation. Brazilian Archives of Biology and Technology 52(special):181-188.

**Rodrigues, C., Vandenberghe, L.P., Oliveira, J., and Soccol, C.R. (2012).** New persepectives of Gibberellic acid production: A review. Critical Reviews in Biotechnology 32(3): 1-11. Doi: 10.3109/07388551.2011.615297.

**Santos, E.M.G., Couto, C.M., Montenegro, M.C., Neves, M.G., Rebelo, S.L., Cavaleiro, J.A.S. and Reis, J.S.** (2003). Ion-selective electrodes based on metallo-pophyrins for gibberellic acid determination in agricultural products. Analytical and Bioanalytical Chemistry 375:511-516.

**Utkarsha, T., Neelam, P. and Nutlam, M.** (2011). Performance of chick pea under the influence of gibberrelic acid and oxygenated peptone during germination. Advances in Bioscience and Biotechnology 2: 40-45. doi:10.4236/abbb.2011.21007