

**MAXIMIZING GIBBERELIC ACID PRODUCTIVITY BY *Fusarium moniliforme* AND ITS EFFECT ON *Saccharomyces* GROWTH**  
**BY**

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**ABSTRACT**

Nutritional and physical fermentation conditions are considerable factors for gibberellic acid production by *Fusarium moniliforme*. Four strains were investigated to produce gibberellic acid, i.e. fermentation process was carried out on three media to select the better one, different concentrations of sucrose, different nitrogen sources, pH levels, temperature range, inoculum size and fermentation period. Also, the effect of gibberellic acid concentrations on *Saccharomyces* (bakers' yeast) was also studied.

Results indicate that Czapek's medium was the best for gibberellic acid production by *Fusarium moniliforme* (F<sub>3</sub>) and (F<sub>1</sub>). *Fusarium moniliforme* (F<sub>3</sub>) was found to be superior in gibberellic acid production than the strain (F<sub>1</sub>).

The maximum yield of gibberellic acid was obtained in the presence of 40 g/L sucrose, NH<sub>4</sub>Cl as a nitrogen source, 2% (v/v) inoculum size, incubation temperature of 25°C, pH 5.5 and 12 days fermentation period.

Results also show that 10 ppm of gibberellic acid is the most effective concentration in increasing the growth of *Saccharomyces*.

**INTRODUCTION**

The gibberellins are a considerable group of hormones that regulate plant growth (Bozhkova *et al.*, 1991; Rachev *et al.*, 1993; Gulewicz *et al.*, 1995; Vanags *et al.*, 1995; Tuomi and Rosenqvist, 1995; Tudzynski, 1999; Gelmi *et al.*, 2000) and have many applications in agriculture and brewing industry (Martin *et al.*, 1995). The gibberellins are obtained industrially from the culture media of *Fusarium moniliforme* (*Gibberella fujikuroi*). It synthesizes different types of gibberellins, the most abundant amongst them is gibberellic acid GA<sub>3</sub>. In addition, GA<sub>3</sub> is also used in a variety of research work and pharmacological application in animals (Kumar and Lonsane, 1990).

Several investigators (Gohlwar *et al.*, 1984; Sunder and Satyavir, 1998; Bruckner and Blechschmidt, 1991) had been reported the importance of

nutritional factors (carbon and nitrogen sources) and physical factors on biosynthesis of such secondary metabolites.

Also, the influence of gibberellic acid on the growth and proliferation of microorganisms has been reported (Fasidi and Olorunmaiye, 1994; Paul *et al.*, 1994; Mohanty and Sethi, 1997).

The purpose of this investigation is to improve the gibberellic acid production conditions and to predict the precise values for different cultural conditions particularly, nitrogen source, carbon source concentration, temperature, pH, inoculum size and fermentation period. The effect of gibberellic acid concentration on the proliferation of *Saccharomyces* (bakers' yeast) as a factor for reducing bread batch fermentation period was also investigated.

### MATERIALS AND METHODS

This investigation was carried out to study the effect of different factors on the gibberellic acid production by *Fusarium moniliforme* strains, i.e. high producer of gibberellic acid, suitable growth medium, suitable concentration of carbon source, suitable nitrogen source, pH level, temperature range, inoculum size and fermentation period.

The effect of gibberellic acid on *Saccharomyces cerevisiae* (bakers' yeast) proliferation as an attempt to diminish bread batch fermentation period was also studied.

#### Source of microorganisms:

Four strains of *Fusarium moniliforme* were obtained from different sources. *Fusarium moniliforme* NBTMCC 349 (F<sub>1</sub>) was obtained from National Bank of Industrial Microorganisms and Cell Cultures, Bulgaria. *Fusarium moniliforme* 52 (F<sub>2</sub>) was obtained from Plant Diseases Dept., Fac. of Agric., Moshtohor. *Fusarium moniliforme* 299 (F<sub>3</sub>) and 305 (F<sub>4</sub>) were obtained from Tissue Culture Lab., Bahtem Agric. Station.

#### Strains maintenance:

*Fusarium* strains were maintained on potato dextrose agar medium at 4°C. For subculturing and propagation, the same medium was used, incubation was carried out for 8 days at 28-30°C.

#### Inocula preparation:

The inocula of *Fusarium* strains were prepared by growing the organisms in glucose medium (Atlas, 1995) for 8 days at 28-30°C on a rotary shaker 240 rpm. Inocula containing of 10<sup>7</sup> spore/ml. were added to the fermentation media in a ratio of 1:10 (v/v).

#### Culture media:

Glucose medium, Czapek's medium and Armstrong medium (Atlas, 1995) were used as gibberellic acid fermentation media to select the best one for

gibberellic acid production. Also, malt extract glucose medium (Shapton and Cooper, 1994) was used for growing *Saccharomyces cerevisiae* (Bakers' yeast) to study the effect of gibberellic acid concentrations on the proliferation of *Saccharomyces cerevisiae*, as well as, the possibility of using gibberellic acid for stimulating the growth of bakers' yeast and consequently reducing bread batch fermentation period.

**Fermentation procedure:**

Shake flasks (500 ml) containing 100 ml fermentation medium were inoculated with 10 ml ( $\times 10^7$  spore/ml) of 8 days old culture inoculum of each of *Fusarium* strains. The shake flasks were incubated on a rotary shaker (240) rpm at 28°C. After fermentation period, the mycelial biomass and liquid phase were obtained to determine mycelial dry weight and gibberellic acid percentage, respectively.

**Mycelial dry weight:**

After fermentation period the growth medium was filtered on weighted Whatman No. 1 filter paper. The fungal biomass and filter paper were dried in an oven at 80°C until reaching a constant weight.

**Gibberellic acid determination:**

Filtrated liquid phase was obtained after fermentation period to determine the gibberellic acid by using spectrophotometer according to Holebrook *et al.* (1961).

Also, reducing sugar was estimated according to Miller (1959).

***Saccharomyces* count:**

The aim of this experiment was to study the effect of different concentrations of gibberellic acid on the counts of *Saccharomyces* for using gibberellic acid in order to reduce the bread batch fermentation period.

Plate count method was used for counting of *Saccharomyces* to determine the most suitable concentration of maximizing *Saccharomyces* proliferation.

## **RESULTS AND DISCUSSION**

**Effect of the different media on gibberellic acid production:**

Data presented in Table (1) show the growth density and gibberellic acid production activity of the four *Fusarium moniliforme* strains on three various media. The maximum production of gibberellic acid was obtained by *Fusarium moniliforme* (F<sub>3</sub>) with different media (232, 384, 217 mg/L) on glucose, czapek's and Armstrong media respectively. This was followed by *Fusarium moniliforme* (F<sub>1</sub>) in its ability to synthesize gibberellic acid. The variation in gibberellic acid production may be due to the variability among *Fusarium moniliforme* strains in biosynthesis of gibberellins (Candau *et al.*, 1991; Sunder and Satyavir, 1998).

Data also indicate that increase in gibberellic acid production and increase in consumed sugar were with Czapek's medium, particularly with *Fusarium moniliforme* (F<sub>3</sub>) and (F<sub>1</sub>). This result may be attributed to the ability of *Fusarium moniliforme* (F<sub>3</sub>, F<sub>1</sub>) to assimilate each of carbon and nitrogen sources and its C/N ratio desirable level (Kumar and Lonsane, 1987; 1990). On the other hand, the lowest production of gibberellic acid and lowest consumption of sugar were observed on the glucose medium with *Fusarium moniliforme* (F<sub>4</sub>). So, the strains (F<sub>1</sub>) and (F<sub>3</sub>) were selected for further studies.

Table (1): Mycelial growth and gibberellic acid production by strains of *Fusarium moniliforme* on different media after 12 days.

Micro-organisms	Mycelial dry weight (g/100ml)	Residual sugar g/100ml	Consumed sugar %	Gibberellic acid mg/L
(Glucose medium)				
F <sub>1</sub>	0.845	0.53	73.5	275
F <sub>2</sub>	0.854	0.86	57.0	206
F <sub>3</sub>	0.962	0.44	78.0	232
F <sub>4</sub>	0.793	1.07	46.5	153
(Czapek's medium)				
F <sub>1</sub>	0.930	0.85	71.6	334
F <sub>2</sub>	0.792	1.18	60.6	246
F <sub>3</sub>	0.986	0.83	72.3	384
F <sub>4</sub>	0.861	1.29	57.0	231
(Armstrong medium)				
F <sub>1</sub>	0.823	0.42	79.0	297
F <sub>2</sub>	0.706	0.91	54.5	193
F <sub>3</sub>	0.885	0.53	73.5	217
F <sub>4</sub>	0.817	0.72	64.0	187

F<sub>1</sub> = *Fusarium moniliforme* NBIMCC 349

F<sub>2</sub> = *Fusarium moniliforme* 52

F<sub>3</sub> = *Fusarium moniliforme* 229

F<sub>4</sub> = *Fusarium moniliforme* 305

#### Effect of carbon source concentration on gibberellic acid production.

Initial concentration of carbon source (sucrose) in basal medium (Czapek's medium) was 30 g/L, which replaced by various concentrations (20, 40, 50 g/L). Data in Table (2) clearly indicate that the maximum production of gibberellic acid was obtained by *Fusarium moniliforme* (F<sub>3</sub>) in the presence of 40 g/L of sucrose. *Fusarium moniliforme* (F<sub>3</sub>) assimilated sucrose more than *Fusarium moniliforme* (F<sub>1</sub>) particularly in the presence of 40 g/L sucrose in fermentation medium (Gancheva and Dimova, 1991). The mycelial dry weight increased and the maximum value observed was in the presence of 40 g/L sucrose for *Fusarium moniliforme* (F<sub>1</sub>) and (F<sub>3</sub>). This result is probably due to the C/N ratio which was the factor that considerably influenced the production of biomass and gibberellic acid, i.e. changes in sucrose concentration led to C/N ratio changes (Escamilla *et al.*, 2000).



Data also indicate that 20 g/L sucrose concentration in fermentation medium gave the minimal production of gibberellic acid. Also, concentration of 50 g/L led to a corresponding decrease in gibberellic acid production. The fact that not only productivity with regard to the substrate consumed, but also absolute production of gibberellic acid, which inferior in the presence of the high level of carbohydrates (Pastrana *et al.*, 1995).

Table (2): Effect of sucrose concentration on gibberellic acid production by *Fusarium moniliforme* after 12 days incubation.

Sucrose concentration (g/L)	F <sub>1</sub>				F <sub>3</sub>			
	Mycelial dry weight (g/100ml)	Residual sugar g/100ml	Consumed sugar %	Gibberellic acid Mg/L	Mycelial dry weight (g/100ml)	Residual sugar g/100ml	Consumed sugar %	Gibberellic acid mg/L
20	0.450	0.72	53.3	205	0.415	0.56	42.7	243
**30	0.863	0.47	82.3	352	0.770	0.38	80.2	361
40	0.905	0.35	87.9	387	0.793	0.30	89.3	397
50	0.720	0.66	78.5	271	0.782	0.53	71.7	288

Refer to footnotes of Table (1).

\*\* Control, 30 g/L sucrose = Initial carbon source concentration in basal medium.

#### Effect of nitrogen source on gibberellic acid production:

Data in Table (3) show that gibberellic acid production ranged from 155 to 363 mg/L. Different nitrogen sources were applied to determine the most suitable one for gibberellic acid production and substituted (on N-basis) with initial nitrogen source in basal medium (0.003% NaNO<sub>3</sub>). Data indicate that the maximum production of gibberellic acid was observed by NH<sub>4</sub>Cl (363 mg/L GA) with *F. moniliforme* (F<sub>3</sub>). Data also indicate that NH<sub>4</sub>Cl was the more effective nitrogen source in gibberellic acid production. This result is in agreement with those obtained by Escamilla *et al.* (2000).

The finding that high gibberellic acid production was achieved with a wide range of nitrogen sources, which demonstrated by Kahlon & Malhotra (1986), and Bruckner & Blechschmidt (1991). On the other hand, the minimal gibberellic acid production was observed in the presence of NH<sub>4</sub>NO<sub>3</sub> with *F. moniliforme* (F<sub>1</sub>). This result may be attributed to that increasing of ammonium concentrations led to suppression of gibberellic acid production (Hollmann *et al.*, 1995).

#### Effect of incubation period on gibberellic acid production

From data recorded in Table (4), it was found that the production of gibberellic acid started from the sixth day and continued to the 12<sup>th</sup> day of incubation. However, the gibberellic acid production continued even at the 12<sup>th</sup> day incubation. The mycelial biomass stopped to increase from the 8<sup>th</sup> day of incubation with both of *F. moniliforme* (F<sub>1</sub>, F<sub>3</sub>). These results are in agreement with those obtained by Kumar and Lonsane (1987). The gibberellic acid production starts to decrease after the 12<sup>th</sup> day. This is due to chemical decomposition and, perhaps, biodegradation of the produced gibberellic acid by the culture itself (Hollmann *et al.*, 1995).

The gibberellic acid production reached its maximum level at the 12<sup>th</sup> day of incubation period. *F. moniliforme* (F<sub>3</sub>) gave gibberellic acid production rate higher than that of *F. moniliforme* (F<sub>1</sub>). The lowest rate of gibberellic acid production was observed at the 3<sup>rd</sup> day of incubation.

Table (3): Effect of nitrogen source on gibberellic acid production by *Fusarium moniliforme* after 12 days incubation.

Nitrogen source (0.003%)	F <sub>1</sub>				F <sub>3</sub>			
	Mycelial dry weight (g/100ml)	Residual sugar g/100ml	Consumed sugar %	Gibberellic acid mg/L	Mycelial dry weight (g/100ml)	Residual sugar g/100ml	Consumed sugar %	Gibberellic acid mg/L
NH <sub>4</sub> NO <sub>3</sub>	0.351	1.19	52.4	155	0.447	1.60	44.3	220
KNO <sub>3</sub>	0.610	0.63	72.5	215	0.710	0.80	42.2	288
NH <sub>4</sub> SO <sub>4</sub>	0.415	0.82	43.6	207	0.620	0.87	36.2	262
NH <sub>4</sub> Cl	0.932	0.42	86.6	352	0.753	0.33	88.3	363
** NaNO <sub>3</sub>	0.802	0.53	82.4	340	0.860	0.42	75.2	352

Refer to footnotes of Table (1).

\*\* Control, NaNO<sub>3</sub> = Initial nitrogen source in basal medium.

Table (4): Effect of incubation period on gibberellic acid production by *Fusarium moniliforme* (F<sub>1</sub> and F<sub>3</sub>)

Incubation Period/ days	F <sub>1</sub>				F <sub>3</sub>			
	Mycelial dry weight (g/100ml)	Residual sugar g/100ml	Consumed sugar %	Gibberellic acid mg/L	Mycelial dry weight (g/100ml)	Residual sugar g/100ml	Consumed sugar %	Gibberellic acid mg/L
0	-	3.0	-	-	-	3.0	-	-
3	0.205	2.4	20.0	67	0.196	2.1	30.0	83
6	0.374	2.0	33.3	109	0.418	1.46	51.3	132
9	0.621	1.31	56.3	243	0.593	1.07	64.3	228
12	0.608	0.7	76.6	364	0.601	0.52	82.6	395
15	0.622	0.4	86.6	329	0.615	0.31	89.6	361

Refer to footnotes of Table (1).

#### Effect of inoculum size on gibberellic acid production:

Data presented in Table (5) show that inoculum levels (1.0, 1.5, 2.0, 2.5% v/v) with a density of 10<sup>7</sup> spore/ml of *F. moniliforme* (F<sub>1</sub>, F<sub>3</sub>), considerably affect gibberellic acid production. An increase of the product was attained in the case of inoculum from 1.0 to 2.0% (v/v). Maximum rate of gibberellic acid was obtained by *F. moniliforme* (F<sub>3</sub>) when 2.0 % inoculum was used in fermentation medium. Data also indicate that the mycelial dry weight increased while the gibberellic acid decreased when 2.5 ml inoculum size was used. This result may be due to the exhaustion of medium components and biodegradation of the product by fungal biomass (Hollmann *et al.*, 1995).

#### Effect of pH level on gibberellic acid production.

The results in Table (6) show the influence of pH on gibberellic acid production by *F. moniliforme* (F<sub>1</sub>, F<sub>3</sub>). Data reveal that the changes in pH levels

was the most important for gibberellic acid production, i.e. the greatest production (416 mg/L) was found at 5.5 pH level with *F. moniliforme* (F<sub>3</sub>). The pH 6.5 and 7.5 decreased gibberellic acid production, while increased the biomass of fungi. These results are in agreement with those obtained by Gohlwar *et al.*, (1984) who mentioned that a maximum yield of gibberellic acid was obtained at 5.5 pH.

**Table (5): Effect of inoculum size on gibberellic acid production by *Fusarium moniliforme* (F<sub>1</sub> and F<sub>3</sub>)**

Inoculum size (v/v)	F <sub>1</sub>				F <sub>3</sub>			
	Mycelial dry weight (g/100ml)	Residual sugar g/100ml	Consumed sugar %	Gibberellic acid mg/L	Mycelial dry weight (g/100ml)	Residual sugar g/100ml	Consumed sugar %	Gibberellic acid mg/L
1.0%	0.735	1.32	56.0	247	0.681	1.18	60.6	285
1.5%	0.942	0.65	78.3	296	0.837	0.52	82.6	310
2.0%	0.955	0.43	85.6	325	0.842	0.36	88.0	348
2.5%	0.990	0.60	80.0	283	0.823	0.42	86.0	312

Inoculum density x 10<sup>7</sup> spore/ ml.

Refer to footnotes of Table (1).

**Table (6): Effect of different pH levels on gibberellic acid production by *Fusarium moniliforme* (F<sub>1</sub> and F<sub>3</sub>)**

pH levels	F <sub>1</sub>				F <sub>3</sub>			
	Mycelial dry weight (g/100ml)	Residual sugar g/100ml	Consumed sugar %	Gibberellic acid mg/L	Mycelial dry weight (g/100ml)	Residual sugar g/100ml	Consumed sugar %	Gibberellic acid mg/L
4.5	0.643	0.84	72.0	179	0.712	0.93	69.0	282
5.5	0.876	0.51	83.0	371	0.852	0.46	84.6	416
6.5	0.890	0.65	78.3	316	0.877	0.58	80.6	382
7.5	0.924	1.01	66.3	246	0.883	1.20	60.0	271

Refer to footnotes of Table (1).

On the other hand, the lowest level of gibberellic acid was observed at pH 4.5 with both fungi.

#### Effect of incubation temperature on gibberellic acid production:

It is obvious from data presented in Table (7) that incubation temperature considerably affects the ability of *F. moniliforme* to produce gibberellic acid. Five different temperatures (15, 20, 25, 30 and 35°C) were used in this investigation. It was found that maximum amount of gibberellic acid (391 mg/L) was produced at 25°C by consumption of 88% sugar. The yield decreased at 15, 20, 30 and 35°C to 216, 293, 283 and 187 mg/L, respectively with *F. moniliforme* (F<sub>3</sub>). This result may probably due to the change in enzyme activity at different temperatures or denaturation of the enzyme at high temperature. According to Kahlon and Malhotra (1986) the proteins fold tightly at lower temperature and thus decrease the catalytic activity.

A similar trend was observed with *F. moniliforme* (F<sub>1</sub>) at different incubation temperature degrees.

Table (7): Effect of incubation temperature on gibberellic acid production by *Fusarium moniliforme* (F<sub>1</sub> and F<sub>3</sub>)

Incubation temperature °C	F <sub>1</sub>				F <sub>3</sub>			
	Mycelial dry weight (g/100ml)	Residual sugar g/100ml	Consumed sugar %	Gibberellic acid mg/L	Mycelial dry weight (g/100ml)	Residual sugar g/100ml	Consumed sugar %	Gibberellic acid mg/L
15	0.376	1.26	58.0	164	0.611	1.52	49.3	216
20	0.519	0.73	75.6	236	0.782	0.89	70.3	293
25	0.925	0.47	84.3	361	1.126	0.36	88.0	391
30	0.807	0.90	70.0	305	0.924	1.04	65.3	283
35	0.536	1.69	43.6	219	0.507	1.84	38.6	187

Refer to footnotes of Table (1).

#### Effect of gibberellic acid on *Saccharomyces* growth.

Data presented in Table (8) show the effect of five concentrations of gibberellic acid (0.1, 1.0, 5, 10, 15 ppm) after 24, 48 and 72 hours of incubation on *Saccharomyces* counts.

Results indicate that the higher and lower concentrations were found to be slightly increased *Saccharomyces* counts. These results are in line with those obtained by Fasidi and Olorunmaiye (1994). The highest counts of *Saccharomyces* were resulted with 10 ppm gibberellic acid concentration.

Table (8): Effect of different concentrations of gibberellic acid on *Saccharomyces* growth

Incubation period (hr)	Count $\times 10^6$ Gibberellic acid concentrations (ppm)					
	Control	0.1	1.0	5.0	10.0	15.0
24	0.86	1.35	1.56	2.34	3.92	2.23
48	1.17	1.82	2.08	2.73	4.29	2.03
72	1.38	1.96	2.25	2.97	4.45	1.93

Initial counts  $35 \times 10^3$  cfu /ml.

This result is nearly similar to that reported by Mohanty and Sethi (1997). Also, Paul *et al.*, (1994) reported that gibberellic acid increased biomass production of the yeast *Kluyveromyces fragilis*. On the other hand, the lowest counts of *Saccharomyces* were resulted with 0.1 and 15 ppm gibberellic acid compared to other treatments. All recorded data emphasized that gibberellic acid increased the biomass production of yeasts. From these data, it can be expect that adding gibberellic acid with bakers' yeast for dough purposes may resulted in shorten the bread batch fermentation period.



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الإنتاجية القصوى لحمض الجبريلليك بواسطة فيوزاريوم مونيليفورم  
وتأثيره على نمو خميرة سكاروميسيس

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تعتبر ظروف التخمر المختلفة من العوامل الهامة لإنتاج حمض الجبريلليك بواسطة فطر *Fusarium moniliforme* خاصة عوامل التغذية والعوامل الطبيعية .

جربت سلالات من فطر *F. moniliforme* لإنتاج حمض الجبريلليك وذلك على ثلاث بيئات غذائية مختلفة لاختيار أحسنها ، على تركيزات متعددة من السكر و كمصدر للكربون ، مصادر نيتروجين مختلفة ، مستويات مختلفة من الـ pH ودرجات الحرارة ، حجم اللقاح ، ومدة التخمر .

وقد كشفت النتائج أن بيئة زابكس كانت احسن البيئات لإنتاج حمض الجبريلليك بواسطة كل من سلالتى الفطر ( $F_1$ ) و ( $F_3$ ) *F. moniliforme*

وكذلك وجد أن سلالة الفطر ( $F_3$ ) *F. moniliforme* تتفوق فى إنتاج حمض الجبريلليك عن السلالة ( $F_1$ ) *F. moniliforme* .

وقد تحقق إنتاج أعلى كمية من حمض الجبريلليك فى وجود ٤٠ جم سكر و / لتر كمصدر كربونى و كلوريد الأمونيوم كمصدر نيتروجينى ركمية لقاح من الفطر ٢% (حجم/حجم) ودرجة حرارة ٢٥ °م و درجة pH ٥,٥ و ذلك بعد ١٢ يوما من النمو على بيئة التخمر .

كما وجد أن لحمض الجبريلليك تأثير فعال فى زيادة أعداد و تكاثر الخميرة (خميرة الخباز) و خاصة عند تركيز ١٠ جزء فى المليون و من النتائج التى تحصل عليها يمكن التوقع بإمكانية إضافة حمض الجبريلليك إلى عجينة الخبز من أجل تقصير فترة تخمر العجين .