Saudi Journal of Biological Sciences 27 (2020) 2431–2438

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Paecilomyces sp. ZB is a cell factory for the production of gibberellic acid using a cheap substrate in solid state fermentation

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ARTICLE INFO

Article history:
Received 30 March 2020
Revised 20 June 2020
Accepted 22 June 2020
Available online 2 July 2020

Keywords:
Gibberellic acid
Response surface methodology
Solid-state fermentation
Plant growth promoter

ABSTRACT

Gibberellic acid from the fungi has been widely used in agriculture. In this study, more than 20 fungal isolates were screened and *Paecilomyces* sp. ZB shown to produce more gibberellic acid than other fungal isolates. Cow dung was used as low cost substrate for gibberellic acid production in solid state fermentation (SSF). Carbon, nitrogen and ionic sources stimulated gibberellic acid production in SSF. Lactose emerged as the significant carbon source supporting more gibberellic acid production (731 μg/g). Among the nitrogen sources, glycine appeared to influence the production of more gibberellic acid (803 μg/g). The process parameters were optimized to enhance gibberellic acid production using a two-level full factorial design and response surface methodology. The amount of gibberellic acid production was influenced mainly by moisture and pH of the substrate. Gibberellic acid production was 1312 μg/g under the optimized conditions and the predicted response was 1339 μg/g. The gibberellic acid yield increased twofolds after medium optimization. The extracted gibberellic acid was sprayed on the growing Mung bean plant and it stimulated the growth of the plant effectively. To conclude, cow dung is a new alternative to produce gibberellic acid in SSF.

1. Introduction

Gibberellic acid is a member of the group of plant growth hormones known as gibberellins. Gibberellic acid is a diterpenoid acid that influences plant growth through stem elongation, germination, breaking dormancy, inducing flowering, sex expression, induction of hydrolytic enzymes, and leaf and fruit senescence (Rodrigues et al., 2012). Hence, gibberellic acid is commercially used to increase the yield in agricultural practices. It is also used in plant tissue culture in low concentrations. There is a huge demand for gibberellic acid in the world market. Due to the price concern, it is used only to improve the yield of selected crops. The substrate such as citric pulp has been used as the cheap biomass for gibberellic acid production (de Oliveira et al., 2017). The fungi *Fusarium moniliforme* is the most used organism for the production of gibberellic acid through submerged fermentation. The yield of gibberellic acid in submerged fermentation is low. Solid-state fermentation (SSF) technology is emerging as an alternative to submerged fermentation in suitable applications. It uses solid substrate with a moisture content of 12–80%. There is no free water since the water retention capacity of the solid substrate retains the moisture within and the final product concentrated (Robinson et al., 2001). Recent reports indicate that gibberellic acid production is much more enhanced in SSF compared with submerged fermentation (Chen, 2013). The possible usage of solid waste materials from agricultural fields and the industries that process agricultural produce makes SSF more attractive. In addition, it is not seasonal and is available throughout the year. Many commercially important products have been produced by SSF using cow dung (Vijayaraghavan et al., 2016). Recently, gibberellic acid production has been optimized for *Fusarium oxysporum* using

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2020.06.040
1319-562X/

various optimization strategies and the potency was evaluated under stress condition (Ben Rhouma et al., 2020). The application of gibberellic acid and advances were recently reported by de Camara et al. (2018).

Factorial design can minimize the number of runs required to find out the significant factors influencing the process and, at the same time, gives information regarding the interaction between the factors (Czitrom, 1999). Furthermore, the runs can be randomized to block the variations within the same levels of a variable (Shivhare and McCreath, 2010). A statistical model in the form of a quadratic equation can be produced using the central composite design (CCD) of response surface methodology (RSM). The vital factors determined using factorial design along with any factors that are generally regarded significant for the process can be included in the CCD process. This method reduces the number of runs required compared with factorial design. After building the statistical model, the same can be used to construct response surface plots and find out the optimal levels of factors to be used in the process (Baş and Boyacı, 2007). The advantage of chief gibberellic acid producing fungus, *Fusarium fujikuroi* and advances were analyzed by Cen et al. (2020). Thin-layer chromatography (TLC) is a cheap and easier technique to compare the gibberellic acid produced through SSF. It can also be used to separate and identify gibberellic acid among the different members of the gibberellin family (Tsavkelova, 2016) and among other plant growth hormones (Karadeniz et al., 2006).

The fungi such as, *Fusarium fujikuroi*, *Fusarium moniliforme*, *Fusarium proliferatum*, and mutant strain of *Fusarium fujikuroi* produced GA3 in submerged and solid state fermentation (Camara et al., 2018). In this paper, cow dung was used as the substrate for SSF for gibberellic acid production using the fungus *Paecilomyces* sp. ZB, which was isolated from the root tip of plantain. The important process parameters of SSF were optimized using RSM. Gibberellic acid was sprayed on Mung bean plant and the efficacy of gibberellic acid on the growth performance was studied.

2. Materials and methods

2.1. Isolation of organisms producing gibberellic acid

The fungus producing gibberellic acid was isolated from a plantain field near Nagercoil, Tamilnadu, India. Samples were collected by swabbing the root tip of banana plants using sterile cotton swabs. The swabs were plated on potato dextrose agar plates of the following composition: [g/l], potatoes 300 g, glucose 20 g, and agar 15 g. Each colony was picked and inoculated into separate nutrient broth liquid media in 250-mL Erlenmeyer flasks.

2.2. Identification of gibberellic acid producing fungal strain

The organism that produced the maximum amount of gibberellic acid among the five isolates was subjected to biochemical characterizations. The fungus was further identified using 18S rRNA sequencing (Rejiniemon et al., 2015). The organism was identified as *Paecilomyces* sp. ZB. The sequence was submitted to GenBank using the BankIt tool.

2.3. Solid-State fermentation and gibberellic acid assay

SSF was carried out in an Erlenmeyer flask of 250 mL by taking 5 g of the substrate and maintaining 70% of moisture using sodium phosphate buffer (pH 6.0, 0.1 M). Five organisms were selected and SSF was carried out individually. The medium components were mixed thoroughly and autoclaved at 121 °C for 15 min. Once the flask reached the room temperature, the medium was inoculated with 0.5 mL of 7-day-old mother culture broth individually under sterile conditions. The flasks were then incubated at 37 °C for 8 days. Then 50-mL sodium phosphate buffer (pH 7.4, 0.1 M) was added to the fermentation medium and mixed well. The slurry was filtered and the filtrate was centrifuged (10,000 rpm, 20 min).

2.4. Quantification of gibberellic acid

50 mL culture extract was filtered using Whatman no 42 filter paper and the pH of the filterate was adjusted at the range of 2.5 – 3.0 using 0.1% HCl. This acidified sample was extracted thrice with ethyl acetate (medium and solvent at 1:3 ratio). The solvent portion of all three stages was collected, dried and residue was suspended in acetonitrile. The residue was used for HPLC analysis. The standard gibberellin (1 mg) GA3 was dissolved in acetonitrile and the final working concentration was adjusted as 1 μg/mL. Standard (10 μL) was injected in HPLC and detected at 206 nm.

2.5. Determination of GA3 using Thin layer chromatography (TLC)

A ready-to-use TLC plate was used for analysis (Merck, Bangalore, India). The TLC plate (10 × 20 cm) was cut appropriately. The sample and standard GA3 was dissolved in acetonitrile and spotted on TLC plates and the solvent was evaporated. Then, the plate was developed inside a TLC chamber using the solvent system [benzene: *n*butanol: acetic acid (6:3:3)]. The developed spots were sprayed with ethanol:conc. sulfuric acid (95:5) and visualized using UV illuminator at 254 nm.

2.6. Initial screening of significant process variables using one-factor-at-a-time (OFAT) approach

SSF was carried out in separate 100-mL Erlenmeyer flasks with 5 g of cow dung substrate. The fermentation process variables such as carbon source (1%) (lactose, sucrose, maltose, glucose, and starch), nitrogen sources (0.5%) (ammonium sulphate, casein, oat meal, glycine, and skim milk), and inorganic salts (0.1%) (calcium, magnesium, manganese, copper, and zinc) were evaluated one at a time, keeping other variables constant. After the completion of fermentation, 50 mL of sodium phosphate buffer (pH 6, 0.1 M) was used to extract the gibberellic acid produced. The experiments were done in triplicate, and the values were averaged.

2.7. Identification of significant process variables using two-level full factorial design

The statistical software Design-Expert 9.0 (StatEase Inc, Minneapolis, MN, USA) was employed to design a full factorial design with two levels of process parameter values. The most significant process variables that influence the production of gibberellic acid were probed by carrying out the experiments according to the design, at two levels (+and -) for the aforementioned five factors and at mid level for other factors. The response Y of the two-level full factorial design is governed by the following first-order polynomial equation:

$$Y = \propto_o + \sum_i \propto_i x_i + \sum_{ij} \propto_{ij} x_i x_j + \sum_{ijk} \propto_{ijk} x_i x_j x_k \quad (1)$$

where α_0 is the intercept, α_i is the ith linear coefficient, and α_{ij} and α_{ijk} are the ijth and ijkth interaction coefficients. The results of the factorial design experiment were fitted in (1). The vitality of the variables or interactions to the process was inferred from the values of coefficients in the resulting polynomial equation. It was further confirmed by performing analysis of variance (ANOVA).

2.8. Building quadratic model using central composite design and finding optimal levels of significant variables using RSM

CCD was used to build a quadratic equation that models the response of most significant factors. Moisture (A), pH (B), and calcium (C) were the factors taken for CCD. These factors were studied at five levels coded as $-\alpha$, -1, 0, 1, and $+\alpha$. The level coded as "0″ was the centre point, "−1" and "1" were the factorial points, and $-\alpha$ and $+\alpha$ were the axial points. The design had 20 experiments involving 8 factorial, 6 axial, and 6 centre points. The experiments were done as triplicates, and the mean value of gibberellic acid amounts is taken as the response Y. The data were fitted in the following quadratic equation for three factors:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{ij=1}^{3} \beta_{ij} X_{ij} \quad (2)$$

where Y is the response, β_0 is the offset term, β_i is the coefficient of linear term, β_{ii} is the coefficient of square terms, and β_{ij} is the coefficient of interactive terms. A, B, and C are represented as X_i's. AB, AC, and BC are represented as X_{ij}'s. The coefficient of the terms reflected their importance in the design.

2.9. Statistical model validation

The optimal levels for the variables for maximum production of gibberellic acid were obtained from RSM. These values were followed in a process, and the response was recorded and compared with the predicted response to validate the model.

2.10. Growth promoting activity of GA on the plant Vigna radiata

In the present study, *Vigna radiata* plant was selected to study the effect of gibberellic acid on the growth. The plant was sprayed with gibberellic acid at the concentration of 50–250 ppm. Double distilled water spray was considered as control. Spraying of plant growth regulators were performed after 15 days of sowing. Manure, water and plant protection measures were taken regularly.

3. Results

3.1. Isolation and screening of gibberellic acid producing the fungus Paecilomyces sp. ZB

Five morphologically different fungal isolates were obtained from banana root tip through the isolation procedure described

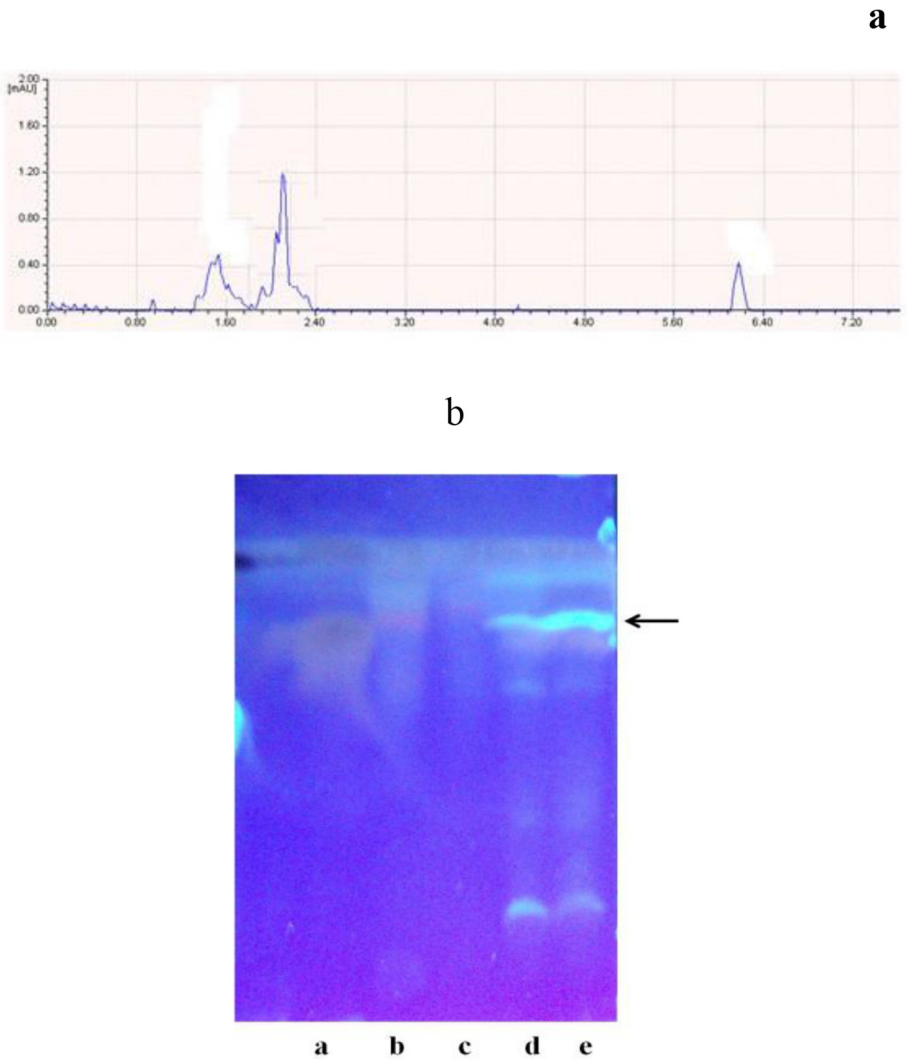

Fig. 1. (a) High Performance Liquid Chromatography separation of gibberellic acid from *Paecilomyces* sp. (b) TLC of gibberellic acid from the five isolated organisms. The medium extract of 20 μL from each of the individual organisms (lanes a–e) were loaded individually. The bands were developed in the solvent system (isopropanol, ammonia, and water, 10:1:1) and the visualized under UV light. Arrow: band corresponding to gibberellic acid. The intense band in lane e indicates the maximum amount of gibberellic acid.

in materials and methods. Gibberellic acid production was found to be maximum in *Paecilomyces* sp. ZB (512 μg/g) among the screened fungal isolates. Through DNA sequencing, the fungus was identified as *Paecilomyces* sp. ZB. The 1630-bp rRNA sequence was submitted to GenBank and accession number was assigned (KX134678).

3.2. Determination of GA3 using High performance liquid chromatography and Thin layer chromatography

In this study, 0.01% *o*-phosphoric acid was used for the elution of GA3 because of the nature of double bond isomer. The elution profile of GA3 using C18 column was achieved at 206 nm (Fig. 1a) with the flow rate of 0. 6 mL per min. The culture supernatant was applied as spots on the TLC plate, and GA3 was visualized under UV light for confirmation. GA3 was appeared as a dark band (Fig. 1b). Before optimization, the yield ranged between 251 and 540 μg/g among the fungal strains and the Fig. 1a showed variation in GA3 yield.

3.3. Traditional OFAT approach revealed lactose, glycine, and calcium as significant carbon, nitrogen, and inorganic ion influencing SSF process

In the present study *Paecilomyces* sp. ZB used cow dung substrate effectively for its growth and gibberellic acid production. The simple "OFAT" approach was used to evaluate the important nutrient sources concerning the SSF using *Paecilomyces* sp. ZB. The crucial physical parameters such as moisture (70%), inoculums size (10%), and fermentation period (7 days) were kept at optimal

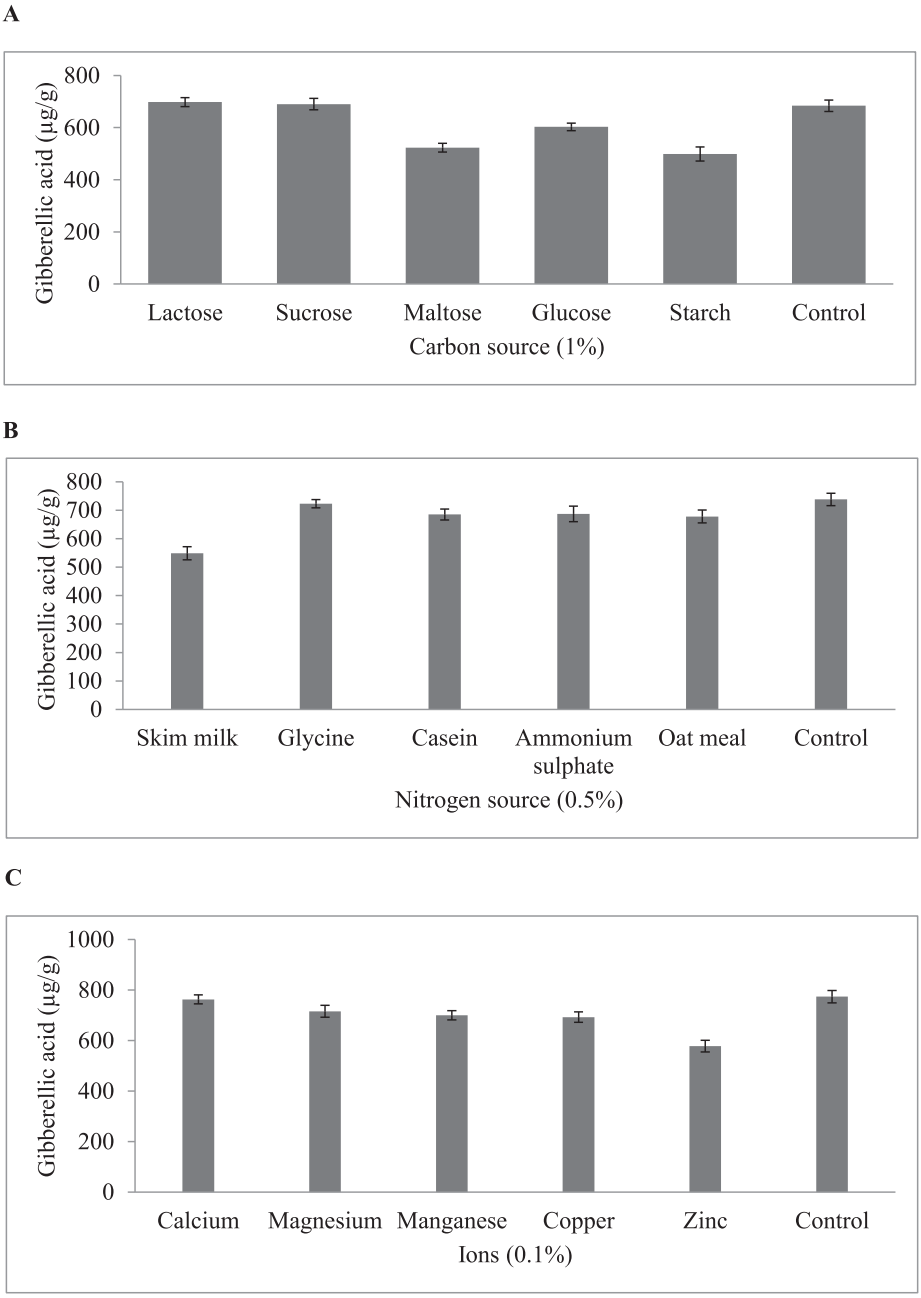

Fig. 2. Effect of various carbon, nitrogen, and inorganic ion sources on gibberellic acid production. The amount of gibberellic acid (μg/g substrate) produced are plotted against the nutrient sources: (a) carbon source, (b) nitrogen source, and (c) inorganic ions. Horizontal bars: standard deviation.

levels during these experimental runs. Glucose, maltose, sucrose, fructose, trehalose, dextrose, and lactose were selected as the candidate carbon sources. Lactose emerged as the significant carbon source supporting more gibberellic acid production (731 μg/g) (Fig. 2A). Among the nitrogen sources, glycine appeared to influence the production of more gibberellic acid (803 μg/g) (Fig. 2B). Calcium in the SSF medium aided the increased production of gibberellic acid (763 μg/g) (Fig. 2C) compared with other ions.

3.4. Two-level five-factor design revealed moisture, pH, and calcium as the most significant factors

The five parameters chosen for two-level full factorial design were A—moisture, B—pH, C—lactose, D—glycine, and E—calcium. The actual levels corresponding to the coded levels were shown in Table 1. The randomized experimental runs and the coded levels of different combinations of the five parameters along with the resultant amount of gibberellic acid produced were shown in Table 2a. The amount of gibberellic acid produced ranged from 1016 to 1470 μg/g cow dung. The results of ANOVA were shown in Table 2b. The model had an F ratio value of 73.74 leaving only less than 0.01% chance for occurrence due to noise. The model terms A, B, E, AC, AD, AE, BC, BD, BE, CD, CE, DE, ABD, ACE, BCD, BCE, ABCD, ACDE, BCDE, and ABCDE were statistically significant. The R^2 value of the model value was 0.9949. The predicted R^2 value was 0.9302, and the adjusted R^2 value was 0.9810. The model had an adequate precision of 35.216, which was way higher than the required value of 4. This makes clear that the signal is adequate, and the model can be used to navigate the design space to predict the relative impact of process parameters. The model equation with significant terms can be written as:

Gibberellic acid (μg/g) = +1237.09 + 20.97A + 63.78B – 7.66E – 10.03AC + 14.34AD – 20.03AE – 11.47BC – 21.34BD – 14.72BE + 19.09CD – 26.78CE – 25.53DE – 15.47ABD + 5.41ABE – 26.41ACE – 6.41ADE – 13.72BCD – 10.84BCE + 26.91ABCD + 19.84ACDE – 9.34 – 31.22ABCDE

The negative coefficient of − 7.66 for the parameter E (calcium) indicated that the amount of gibberellic acid produced could be increased by decreasing the amount of calcium in the medium. Based on the results of two-level full factorial design, moisture, pH, and calcium were considered for CCD and RSM.

3.5. CCD and RSM

The three vital process parameters moisture (A), pH (B) and calcium (C) were taken at five coded levels (−α, −1, 0, +1, and + α), as shown in Table 3. ANOVA was performed on the results of CCD, and the following quadratic equation was obtained:

Gibberellic acid (μg/g) = +1335.98 + 23.08A + 22.20B − 2.91C – 14.75AB + 33.25AC – 13.50BC 39.51A^2 – 42.16B^2 − 32.61C^2.

Gibberellic acid production observed was maximum (1360 μg/g) at run 16 (Table 4a). The model F-value was 30.79, and this implied that the model was statistically significant (Table 4b). There was only 0.01% chance that this much large F-value could occur due to noise. The model terms A, B, AB, AC, BC, A^2, B^2, and

Table 1
The independent variables chosen for 2^5 factorial design and their coded levels.

Symbol	Variables	Units	Coded levels	
			−1	+1
A	Moisture	%	60	90
B	pH		5	7
C	Lactose	%	0.1	1
D	Glycine	%	0.1	0.5
E	Calcium	%	0.05	0.5

Table 2a
Randomized runs of 2^5 factorial design and gibberllic acid production.

Run	A:Moisture %	B:pH	C:Lactose %	D:Glycine %	E:Calcium %	GAA (μg/g)
1	1	1	1	1	1	1163
2	−1	−1	−1	1	−1	1033
3	1	1	−1	−1	1	1442
4	−1	1	−1	1	−1	1288
5	−1	1	−1	1	1	1264
6	1	−1	−1	−1	−1	1021
7	1	1	−1	1	1	1303
8	−1	1	−1	−1	−1	1241
9	1	1	1	−1	1	1246
10	1	−1	1	1	−1	1292
11	1	−1	1	−1	1	1043
12	−1	−1	1	1	1	1209
13	1	1	−1	−1	−1	1365
14	−1	1	1	1	1	1204
15	−1	1	1	−1	−1	1332
16	1	1	1	1	−1	1470
17	−1	1	−1	−1	1	1290
18	−1	−1	−1	−1	1	1200
19	1	−1	−1	1	−1	1290
20	1	−1	−1	1	1	1164
21	−1	−1	1	−1	1	1235
22	1	−1	1	−1	−1	1233
23	1	−1	1	1	1	1228
24	1	1	1	−1	−1	1332
25	−1	1	1	1	−1	1275
26	−1	1	1	−1	1	1316
27	1	−1	−1	−1	1	1254
28	1	1	−1	1	−1	1283
29	−1	−1	1	−1	−1	1016
30	−1	−1	1	1	−1	1245
31	−1	−1	−1	1	1	1110
32	−1	−1	−1	−1	−1	1200

Table 2b
ANOVA results for 2^5 factorial design for screening of selected variables.

Source	Sum of squares	df	Mean square	F-Value	p-Value
Model	3.617E + 005	22	16441.63	73.74	< 0.0001
A-Moisture	14070.03	1	14070.03	63.10	< 0.0001
B-pH	1.302E + 005	1	1.302E + 005	583.82	< 0.0001
E-Calcium	1875.00	1	1875.00	8.41	0.0176
AC	3220.50	1	3220.50	14.44	0.0042
AD	6583.13	1	6583.13	29.53	0.0004
AE	12840.13	1	12840.13	57.58	< 0.0001
BC	4209.13	1	4209.13	18.88	0.0019
BD	14577.78	1	14577.78	65.38	<0.0001
BE	6932.53	1	6932.53	31.09	0.0003
CD	11666.13	1	11666.13	52.32	< 0.0001
CE	22951.13	1	22951.13	102.93	< 0.0001
DE	20859.50	1	20859.50	93.55	< 0.0001
ABD	7657.13	1	7657.13	34.34	0.0002
ABE	935.13	1	935.13	4.19	0.0708
ACE	22313.50	1	22313.50	100.07	< 0.0001
ADE	1313.13	1	1313.13	5.89	0.0382
BCD	6022.13	1	6022.13	27.01	0.0006
BCE	3762.13	1	3762.13	16.88	0.0026
ABCD	23166.28	1	23166.28	103.90	< 0.0001
ACDE	12600.78	1	12600.78	56.51	< 0.0001
BCDE	2793.78	1	2793.78	12.53	0.0063
ABCDE	31187.53	1	31187.53	139.87	< 0.0001
Residual	2006.78	9	222.98		
Cor Total	3.637E + 005	31			

Table 3
The independent variables selected for CCD and their coded values.

Variables	Symbol	Coded values				
		-α	−1	0	+1	+ α
Moisture	A	49.7731	60	75	90	100.227
pH	B	4.31821	5	6	7	7.68179
Calcium	C	−0.206807	0.1	0.55	1	1.30681

Table 4a
CCD experimental runs for optimizing gibberellic acid production.

Run	A:Moisture	B:pH	C:Calcium	GAA (μg/g)
1	0.000	0.000	0.000	1348
2	1.682	0.000	0.000	1260
3	−1.000	1.000	−1.000	1291
4	1.000	−1.000	1.000	1280
5	−1.000	−1.000	−1.000	1187
6	0.000	0.000	1.682	1240
7	1.000	1.000	−1.000	1247
8	0.000	1.682	0.000	1240
9	0.000	0.000	0.000	1340
10	−1.000	−1.000	1.000	1132
11	1.000	1.000	1.000	1271
12	0.000	0.000	0.000	1321
13	0.000	0.000	0.000	1302
14	0.000	−1.682	0.000	1187
15	0.000	0.000	−1.682	1241
16	0.000	0.000	0.000	1360
17	1.000	−1.000	−1.000	1190
18	−1.682	0.000	0.000	1182
19	−1.000	1.000	1.000	1194
20	0.000	0.000	0.000	1346

Table 4b
ANOVA for CCD design results.

Source	Sum of squares	df	Mean square	F-Value	p-Value
Model	79274.50	9	8808.28	30.79	<0.0001
A-Moisture	7273.88	1	7273.88	25.43	0.0005
B-pH	6728.55	1	6728.55	23.52	0.0007
C-Calcium	115.30	1	115.30	0.40	0.5398
AB	1740.50	1	1740.50	6.08	0.0333
AC	8844.50	1	8844.50	30.92	0.0002
BC	1458.00	1	1458.00	5.10	0.0476
A^2	22491.56	1	22491.56	78.63	<0.0001
B^2	25612.20	1	25612.20	89.54	<0.0001
C^2	15326.34	1	15326.34	53.58	<0.0001
Residual	2860.45	10	286.05		
Lack of fit	643.62	5	128.72	0.29	0.8996
Pure error	2216.83	5	443.37		
Cor total	82134.95	19			

C^2 were concluded as significant as their "Prob > F" value is <0.05. In addition, the model does not have a significant lack of fit F-value (0.29) relative to the pure error. In this model, the predicted R-squared value (0.9003) is in good agreement with the adjusted R-squared value (0.9338). The optimal level of moisture is 79%, pH is 6.2, and calcium is 0.01%. The three-dimensional response surface graph depicts the interaction between the variables and maintains the third variable at its zero level. As shown in Fig. 3, the interactions between pH and moisture content variable were found to be significant. Gibberellic acid production of the SSF varied significantly upon the changes in pH and moisture.

3.6. Validation of the statistical model

The predictive model obtained through CCD was validated by carrying out the fermentation process in triplicates. Cow dung substrate along with the optimized levels of process parameters was used in the process, and the amount of gibberellic acid produced was estimated. The amount of gibberellic acid produced was 1312 μg/g, which was in agreement with the predicted amount

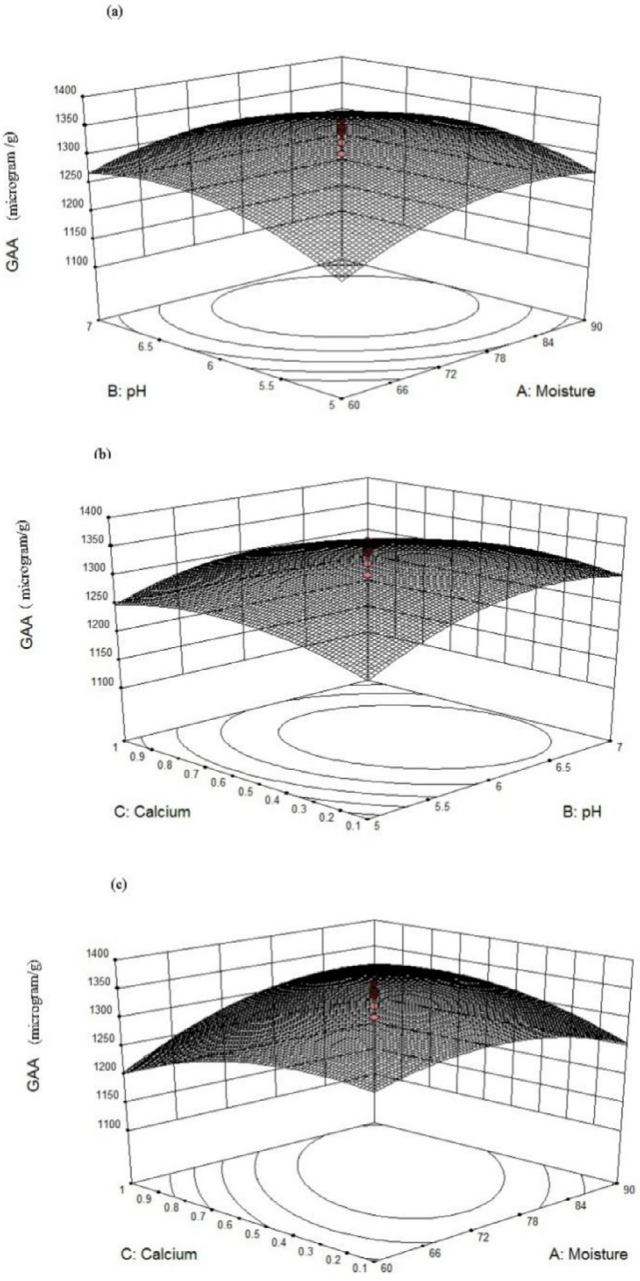

Fig. 3. Three-dimensional response surface plots. (a) Response surface showing the interactive effect of pH and moisture on gibberellic acid production in SSF using cow dung employing *Paecilomyces* sp. ZB. (b) Response surface showing the interactive effect of calcium and moisture on gibberellic acid production in SSF using cow dung employing *Paecilomyces* sp. ZB. (c) Response surface showing the interactive effect of calcium and pH on gibberellic acid production in SSF using cow dung employing *Paecilomyces* sp. ZB.

of 1339 μg/g. Thus, there was a two-fold increase in the production of gibberellic acid by *Paecilomyces* sp. ZB using cow dung substrate.

3.7. GA induced growth on the plant Vigna ratiata

Plant height reached 17 ± 3.7 cm in the treatment group containing 50 ppm GA spray. However, it reached 33.5 ± 6.1 cm and 40.1 ± 4.2 cm in the treatment group containing 100 and 150 ppm. Maximum plant height was measured as 44.5 ± 1.7 cm in 200 ppm spray. Moreover, at 250 ppm GA spray, plant height was not increased much compared with previous GA concentration (Fig. 4).

Fig. 4. Growth of Mung bean plant treated with gibberellic acid from *Paecilomyces* sp. ZB (a) Plant treated with commercial gibberellic acid and (b) plant treated with *Paecilomyces* sp. ZB gibberellic acid.

4. Discussion

SSF is widely used for the production of gibberellic acid. In SSF, the solid wastes/agro industrial residues are frequently used for gibberellic acid production. Machado et al. (2002) applied coffee husk and cassava bagasse for gibberellic acid production by *Gibberella fujikuroi* and *Fusarium moniliforme*. Wheat gluten medium was applied for the production of gibberellic acid by *Gibberella fujikuroi* (Lale and Gadre, 2010). Cow dung substrate was earlier used for the production of various biomolecules (Vijayaraghavan et al., 2016); however, the production of gibberellic acid was not reported. Here for the first time, we report the use of cow dung for the production of gibberellic acid. This substrate was recognized as a cheap material and it was used as substrate for gibberellic acid production by *Paecilomyces* sp. ZB. In SSF, production of gibberellic acid ranges from a few μg to 8 mg / g of dry substrate (Rodrigues et al., 2016). In this study gibberellic acid production was 5.12 mg/g before optimization, which was maximum among the selected fungal isolates. Machado et al. (2000) screened gibberellic acid producing fungi and used coffee husk as the substrate and the achieved yield was 100 mg/kg.

In recent years, increased attention has been paid for the production of gibberellic acid using statistical approach. Rodrigues et al. (2009) employed the low-cost substrates, such as citric pulp, soy bran, soy husk, sugarcane bagasse, coffee husk, and cassava bagasse, for gibberellic acid production by *Fusarium moniliforme* using central composite rotatory design. Likewise, Isa and MatDon (2014) optimized the culture conditions for gibberellic acid production in SSF by Box-Behnken design, and the yield was 31.57 μg/g substrate. In our study the optimized medium showed 1312 μg/g, which was found to be good than certain fungal species. In comparison with other fungal species, *Paecilomyces* sp. ZB offers good options for gibberellic acid production, because it uses a cheap substrate for gibberellic acid production. The production of gibberellic acid by SSF allows the recycling of cow dung, with reduced production costs.

GA is one of the plant growth regulators which promoted plant growth in higher plants, but is also produced by bacteria and fungi (MacMillan, 2001). The initial step of GA biosynthesis pathways reported in fungi is almost similar with plants (Chanclud and Morel, 2016). It was previously reported that the application of GAs on the plants promote cell division (Arteca, 1996) and cell enlargement (Liu and Loy, 1976). These were applied on plants to enhance the growth and reported stimulated effect in Chinese cabbage, cucumber and crown daisy. In the present study, 200 ppm gibberellic acid spray concentration was maximum and enhanced plant growth (74.5 ± 6.7 cm). At higher GA concentration, plant growth suppressed. GA increased number of branches, plant height, number of leaves, leaf area, dry and fresh weights (Khan et al., 2010). In dwarf pea seeds, application of GA enhanced shoots growth stimulation and was reported (Baumgartner et al., 2008). In Faba Bean, the combination of 20 mM Ca^{+2} with 10^{-6} M GA3 increased shoot fresh weight, plant height, shoot dry weight, root fresh weight, root length, root number, root dry weight, water content, anthocyanin, chlorophyll and carbonic anhydrase activity (Al-Whaibi et al., 2010).

5. Conclusions

In conclusion, GA is a good plant growth regulator with numerous valued applications in agriculture sector. The industrial process presently used to produce GA is mainly based on submerged fermentation using *Fusarium moniliforme* or *Gibberella fujikuroi*. In this context, SSF fermentation technique has numerous advantages over submerged fermentation process for the production of metabolites and valorization of agro-wastes that can be used as cheap substrates.

Acknowledgements

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group no. (RGP-1438-053).

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