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Research Article

PRODUCTION AND BIOASSAY EVALUATION OF GIBBERELLIC ACID USING *FUSARIUM MONILIFORME*

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ABSTRACT
Gibberellic acid is an important plant growth regulator widely used in agriculture for enhancing plant growth, seed germination, flowering, and fruit development. The present study investigated the production of gibberellic acid by *Fusarium moniliforme* NCIM 1099 using surface and submerged fermentation techniques. The organism was cultivated in optimized production media under controlled conditions and evaluated for gibberellic acid production. Thin-layer chromatography analysis showed an Rf value of 0.62 corresponding to the standard, while HPLC analysis confirmed gibberellic acid production with retention times comparable to the standard. Comparative fermentation studies revealed its higher production under submerged fermentation (424 µg/mL) than surface fermentation (370 µg/mL), with corresponding yields of 0.16 and 0.14 mg/100 mL, respectively. Growth-promoting activity of the extracted gibberellic acid was demonstrated using *Pisum sativum* seedlings. The study highlights the efficiency of submerged fermentation for producing biologically active gibberellic acid using *Fusarium moniliforme* and its potential applications in agricultural biotechnology.

Keywords: *Gibberellic acid, Fusarium moniliforme*

INTRODUCTION

Biologically active endogenous phytohormones regulate several physiological and developmental processes in plants, including stem elongation, root proliferation and seed germination (Pandya *et al*., 2023). It is also reported to participate in processes of flowering and fruit expansion (Hernández Rodríguez *et al*., 2024; Vishal & Kumar, 2018). Due to their significant role in plant growth and productivity, they have been widely used in agriculture to improve crop yields (Castro-Camba *et al*., 2022). The response of crops to Gibberellic acid treatment varies with crop species and application method. For instance, spraying treatments on grapes enhanced berry size and facilitated harvest control, while in oranges, they were effective in regulating harvest (Coggins *et al*., 1966). Seed soaking treatments improved malting quality in barley and reduced dormancy in potato seeds. Similarly, foliar spraying delayed flowering in mango, providing advantages in crop management. Gibberellic acid application increased yield and fruit growth in banana, pea and tomato (Pramanik *et al*., 2017).

The presence of gibberellic acid in higher plants has been identified in several plant species, including maize, wheat, barley, peaches, spinach, and onions (Kalra & Bhatla, 2018). Biosynthesis primarily occurs in seedlings, young leaves, root tips, flower parts, mature seeds, and germinating embryos (Gupta& Chakrabarty, 2013). Despite their importance, Gibberellic acids are naturally synthesized in very low concentrations in plants, making extraction costly and commercially impractical. Chemical synthesis is also economically unfavourable because it involves multiple reaction steps and expensive reagents. Increasing global demand in agriculture has encouraged the exploration of microbial sources for Gibberellic acid production. Its production was first associated with the fungus *Gibberella fujikuroi* and has since been reported in several fungi, including *Fusarium moniliforme*, *Fusarium oxysporum, Aspergillus flavus, and Neurospora crassa* (Hernández Rodríguez *et al*., 2024). Certain bacteria, such as *Azotobacter chroococcum, Agrobacterium radiobacter, Bacillus licheniformis, Bacillus pumilus,* and *Pseudomonas fluorescens,* have also been reported to produce Gibberellic acid (Desai, 2017; Rademacher, 1994). Its microbial production

is influenced by nutritional, physical, and fermentation parameters. Carbon sources such as glucose and sucrose are commonly used, whereas nitrogen sources include ammonium sulfate, ammonium chloride, glycine, ammonium tartrate, and corn steep liquor. The carbon-to-nitrogen ratio plays a critical role. In earlier work, studies were conducted to optimise the C:N ratio using coffee husk and cassava bagasse, both of which contain amide groups (Machado *et al*., 2002). Production methods include surface fermentation, liquid surface fermentation, submerged fermentation, and solid-state fermentation, each having distinct advantages and limitations (Hernández Rodríguez *et al*., 2024; Rodrigues *et al*., 2012). Among these, submerged fermentation is considered economically viable and suitable for large-scale industrial production, whereas solid-state fermentation offers operational simplicity and scalability. In recent years, solid-state fermentation (SSF) with low-cost agro-industrial by-products has been used as a substrate (Pinheiro *et al*., 2024; El-Sheikh *et al*., 2020).

MATERIALS AND METHODS

Materials

All ingredients used for the preparation of media/chemicals were A.R. grade and sourced from Hi-media Ltd, Mumbai; Loba Chemie Ltd, Mumbai; and Qualigens. The microbial strain used for Gibberellic acid production was *Fusarium moniliforme* NCIM 1099 (ATCC12616). It was subcultured on potato dextrose agar and maintained at 4°C.

Production Media

Gibberellic acid production was carried out using Surface and submerged fermentation media. The composition of Liquid surface fermentation media includes (g/L) Glucose (0.0025), $MgSO_4 \cdot 7H_2O$ (0.005), KH_2PO_4 (0.005), $FeSO_4 \cdot 7H_2O$ (0.02), $ZnSO_4 \cdot 7H_2O$ (0.002), $CaCl_2 \cdot 2H_2O$ (0.01), $CuSO_4 \cdot 5H_2O$ (0.002), $Na_2B_4O_7 \cdot 10H_2O$ (0.002), $Na_2MoO_7 \cdot 2H_2O$ (0.002), $MnSO_4 \cdot H_2O$ (0.002), Ethylenediaminetetraacetic acid (EDTA) (0.6) and Submerged fermentation media content (g/L) Glucose (0.25), ammonium nitrate (0.14), Al_2O_3(0.05), $ZnCl_2$ (0.05), $CuSO_4 \cdot 7H_2O$(0.01), $KH_2PO_4 \cdot 10H_2O$(0.05), $MgSO_4$(0.02), and K_2SO_4 (0.06) (Camara *et al*., 2018; Rodrigues *et al*., 2012; Sanchez-Marroquin, 1963).

Production and recovery

The mycelial culture of *Fusarium moniliforme* NCIM 1099 was transferred aseptically into 100 mL of seed medium containing sterile Potato Dextrose Broth and incubated for 72 h. Subsequently, the grown culture was transferred to a similar medium and incubated for an additional 48 h on a rotary at 120 rpm to obtain active fungal biomass. 10% (v/v) of mycelial inoculum was aseptically transferred into the sterilized production medium and incubated for 7 days at room temperature. In submerged fermentation, the medium was incubated on a rotary shaker at 120 rpm and 28°C. After incubation, the medium was subjected to gibberellic acid recovery.

The fermentation broth was filtered and centrifuged at 2000 rpm for 10 min to obtain a cell-free supernatant. The clarified supernatant was then treated with activated charcoal to adsorb gibberellins. The adsorbed compounds were subsequently eluted using acetone, and the eluate was concentrated under reduced pressure to obtain an aqueous concentrate. Further purification was carried out by extraction with 1% sodium carbonate (Na_2CO_3) solution. The extract was acidified to pH 3.0 using 0.1 N hydrochloric acid and then subjected to solvent extraction with ethyl acetate. Finally, the solvent phase was evaporated under reduced pressure, followed by concentration and crystallization to obtain purified gibberellic acid crystals (Adejoro *et al.,* 2018; Sharma *et al*., 2018). To confirm the purity, the compound's melting point was determined by using Thiele's method.

Detection

Gibberellic acid was detected by TLC using an isopropanol: water (80:20) solvent system and 5% H_2SO_4 in ethanol as the spraying reagent; the results were compared with the standard. The results were also confirmed by examining the TLC plate under U.V. at 250 nm. The fermentation broth was pretreated and analyzed using an HPLC system equipped with a UV-100 detector. The column used was Capcellpak C18 (4.6mm x 250mm; 5 pm particle size). The mobile phase contained 35% methanol and 65% water adjusted

to pH 3.0 with phosphoric acid (Sharma *et al*., 2018; Machado *et al*., 2002). The flow rate was controlled at 1.0 ml/min. The analysis was done using WS-100 workstation software. The quantitative estimation of Gibberellic acid was performed by spectrophotometric analysis at 650nm using the Phosphomolybdic acid (ml) reagent.

Bioassay of Gibberellic acid

The growth promotion ability of recovered gibberellic acid was performed with seeds of *Pisum sativum* (Pea). The seeds were treated with recovered gibberellic acid and sown in the black soil. The seeds were watered at a regular interval and kept under controlled conditions for 2 weeks. The length of the seedling is measured in centimetres. The results were also recorded by observing seedling growth under similar conditions with standard gibberellic acid.

RESULTS & DISCUSSION

The production of gibberellic acid by *Fusarium moniliforme* was evaluated under submerged and surface fermentation conditions. The initial sugar concentration in both fermentation systems was maintained at 6.2 mg/mL. At the end of fermentation, the analysis of residual sugar indicated 81.87% and 84.19% utilization of surface and submerged fermentations, respectively. The results indicate greater substrate utilization under submerged fermentation conditions. Submerged fermentation showed comparatively higher production than Surface fermentation (Table 1).

Table 1: Results of gibberellic acid production using surface and submerged fermentation

Observations	Surface	Submerged
Initial sugar content (mg/ml)	6.2	6.2
Residual sugar content (mg/ml)	1.124	0.980
Sugar utilized (mg/ml)	5.076	5.22
Gibberellin content (µg/ml)	370	424
% Yield (mg/100 ml)	0.14	0.16

These findings suggest that submerged fermentation was more efficient for gibberellic acid production by *Fusarium moniliforme* under the conditions studied, yielding 424 µg/ml. The results are consistent with previous reports (Sharma *et al.,* 2018), in which fluorescent *Pseudomonas* produced the plant growth regulator gibberellic acid and were directly involved in the growth and development of plant roots and shoots. The positive effect of adding glucose was highlighted in earlier reports (Pinheiro *et al*., 2024). The melting point of extracted gibberellin was 230± 2°C and Rf value of 0.62, which corresponded closely with the standard, confirming the presence of gibberellic acid in the sample.

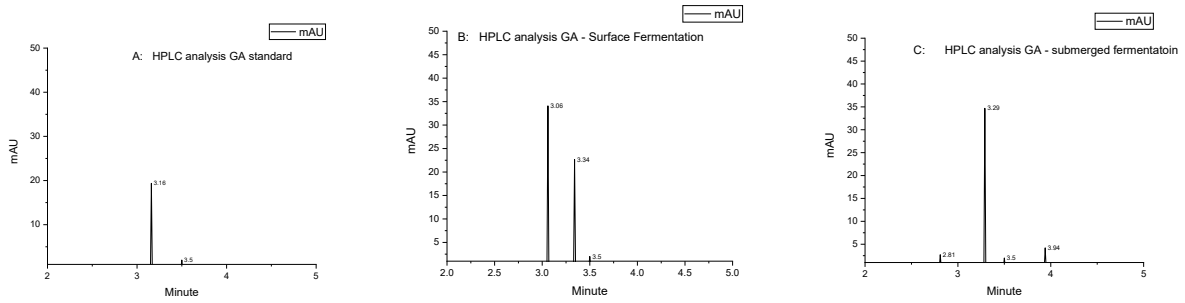

Figure 1: HPLC analysis of Gibberellic acid
(A: Standard; B: production using surface fermentation; C: production using submerged fermentation)

High-Performance Liquid Chromatography (HPLC) analysis has confirmed the presence and purity of gibberellic acid produced during fermentation. The extracted sample was analyzed along with a standard solution under identical chromatographic conditions. The chromatogram of the sample showed prominent peaks at 3.29 and 3.06 for submerged and surface fermentations, respectively. This corresponds to a 3.18 retention time of standard gibberellic acid, thereby confirming the production by *Fusarium moniliforme* (Figure 1). The retention time in the present study was comparable to that of previously reported studies (Baliyan *et al*., 2022; Sharma *et al*., 2018).

Bioassay of gibberellic acid

The growth promoting effect of recovered gibberellic acid was evaluated based on seedling elongation response. The untreated control seedlings showed an induced length of 19.5± 1.3 cm, whereas seedlings treated with standard gibberellic acid exhibited a higher elongation of 31.6± 2.3 cm. The test sample produced a seedling length of 26.4±1.2 cm, indicating substantial growth promotion activity compared to the control. The increase in seedling length observed in the test sample confirmed the presence of biologically active gibberellic acid in the fermented extract. The growth response was comparable to that of the standard treatment. Similar notable results for growth promotion were reported previously for Gibberellic acid using *Cicer arietinum* (Chickpea) (Baliyan *et al*., 2022).

CONCLUSION

The present study demonstrated the production of gibberellic acid by *Fusarium moniliforme* NCIM 1099 via both surface and submerged fermentation. Comparative evaluation of the fermentation methods revealed that submerged fermentation was more efficient, yielding a higher concentration (424 µg/mL) than surface fermentation (370 µg/mL). Qualitative characterization by TLC, HPLC, and melting point determination confirmed the identity and purity of the gibberellic acid produced. The recovered gibberellic acid exhibited enhanced seedling length of *Pisum sativum* as compared with the control. These findings establish *F. moniliforme* as a promising microbial source for the economical production of biologically active gibberellic acid.

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