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Improved Production of Ga3 on Cheap Novel Substrate by *Fusarium Moniliforme*

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Abstract: The study shows that Solid state fermentation technique leads to a better production of Gibberellic acid from the fungi Fusarium Moniliforme 1100. The total yield of the Gibberellic acid obtained was 5%. We are reporting a new commercially viable improved low-cost process using cheap substrates for increased production of Gibberellic acid from fungi

Keywords: Optimization, Large-scale Solid-State Fermentation, Gibberellic acid, Citric Peel.

I. INTRODUCTION

Gibberellins (gas) are plant hormones that regulate growth by its effect on cell growth and cell elongation. Such effects are often seen in stem growth as well as root growth. Stems and internodal lengths can be increased and a better more extensive root system develops. Increases in cell division can also sometimes be seen in the production of larger leaves. Ga leads to bigger plants with bigger shoots and leaves in many plants. Longer flower stems can be produced on a range of 'florist' flower crops. In addition, flower size can sometimes be achieved. Treating flower buds before opening with a solution of gibberellic acid can lead to huge flower increases in a range of different flowers which is of commercial impotence's currently, there are 136 gibberellins (gas) isolated from plants, produced by microorganisms such as fungi and bacteria or obtained synthetically (jeers, 1970; Blake, Taylor and crisp, 2000; Hedden and Phillips, 2000; bömke and Tudzynski, 2009). Gibberellins are designated by gan where "n" corresponds, approximately, to the order of its discovery (hill, 1977; Arteca, 1995) chemically, ga3 is a tetracyclic dihydroxy γ-lactonic acid containing two ethylene bonds and one free carboxylic acid group. Its chemical structure (c19h22o6) is presented in figure 1 (mander, 1992)

II. MATERIALS AND METHODS

II.I Micro Organism-Organism and Growth Conditions *Fusarium Moniliforme* 1100 was obtained from National Collection of Industrial Microorganisms India. The strain was cultured and maintained on Potato dextrose agar (PDA) slants.

II.II Substrate- The Dry Orange Peel (Waste) and its outer cover are dried and ground to form Course powder.

II.III PDA Medium plate- Potato Dextrose Agar media was prepared by adding, 4 gram PDA dissolved in 100 ml of distilled water in a 250 ml Erlenmeyer flask. Then PDA media was autoclaved at 121° C at a pressure of Pascal's PSI for 15 minutes to avoid contamination. PDA plates were prepared and allowed for solidification in laminar air flow.

II.IV Culture of *Fusarium moniliforme*- The inoculum used was active Glycerol preserved pure cultures and inoculation was done over PDA plate to culture *"Fusarium moniliforme*" and incubated in B.O.D. incubator at 25° C for 8 days. The mycelia of the selected fungus were produced in 250 ml Erlenmeyer flasks containing 100 ml PD broth medium from the stock culture and incubated at 25ºC for Five days. The obtained suspension was then stored at 4ºC for up to seven days.

II.V. Inoculum preparation for Solid Substrate Fermentation -For the preparation of inoculum, the fungus was grown in 2000 ml Erlenmeyer flasks containing 1300 ml PDB broth at 25℃ for 5 days. Growth was monitored every 24 h.

II.V.I Medium preparation for Solid Substrate Fermentation of *Fusarium Moniliforme* 1100- For SSF media was prepared using citric pulp powder 4.5 Kg of the powder was mixed with 135 gm glucose ($C_6H_{12}O_6$) and ammonium sulfate $(NH_4)_2SO_4$ as ions in 21.6 m/liter concentration was added. The medium was mixed with water to make a thick slurry in 9L water. The pH was maintained at 5.5. The initial moisture content of the medium was adjusted to 60% and then transferred the Semisolid substrate media into plates of size 10 cm diameter, the plates were covered with autoclave able poly bag and then sterilized in an autoclave maintained at 15 psi for 20 min and temperature at 121 ° C upon cooling.

II.V.II Inoculation in the medium plates- The sterile production medium was inoculated with 25 ml of a 5-day old inoculum of *Fusarium moniliforme*, 1100 in each plate and was mixed thoroughly. It was incubated at 25℃ for 8 days. A cultured plate of *Fusarium Moniliforme 1100 was Showed* light pink color cottony colonies over the medium substrate.

II.V.III Extraction of GA3 from the SSF -All the 300 plates were harvested, from this 100 plates culture was crushed in 10-liter butanol and then transferred to 10 L capacity drum and kept at shaking for 8 hrs. After filtration 10 liters extracted Butanol layer was obtained. To this, another set of 100 splate's culture was added to perform second cycle and third cycle simultaneously. The filtered butanol extract was kept in cold condition and then it was taken for analysis and quantification of GA3 % in butanol extract.

II.V.IV HPLC of the extracted secondary metabolite in butanol with GA3 as a standard-For identification of the secondary metabolite present in the butanol HPLC with the standard GA3 was done. For the preparation of standard 200 mg GA3acid powder was dissolved in 50 ml methanol of HPLC methanol (4mg/ml) then form this 1 gm (4mg) was mixed in 9 ml of HPLC methanol the final concentration was 0.4%/ml.For the preparation of crude extracted gibberellins – 1gm of the sample was mixed with 9 ml of HPLC methanol volume makeup to 10 ml in methanol. The sample was injected using a Rheodyne injector with 20 ul loop. GA3 was estimated by HPLC at 206 nm on a c18 column using acetonitrile: HPLC Methanol 1:1 Ratio as the mobile phase at 1 ml/ minute flow rate.

II.V.V Calculation to determine weight of sample

$$\frac{\text{Peak area of Sample}}{\text{Peak area of Standard}} \times \frac{\text{Weight of STD}}{\text{Weight of Sample}} \times \text{Purity of STD}$$

II.VI Formulation Preparation- For the plant experiment formulation was prepared by using different concentrations. 25 µl crude extracted butanol layers were taken. 22.5 ml solvent and 2.5 emulsifiers were mixed to the extracted GA3 The formulation was stored at room temperature and used as per experimental protocol on pots experiment and results we noted down and analysis for further studies.

II.VI.I POT Trails to study- The Effect of GA3 on major agriculture crop chickpea and green gram of mp region a pot trails experiment was performed using two sets of 6 pots to shown seeds of chickpea and green gram. The trail was designed to have negative control, positive control and the experimental pot (test Control) which was spared with5 % ga3 formulation the positive control was spared with chamatkar (Common plant PGPR available in local market) the experiment was conducted for 24 days and the pots were spared with ga3 formulations at least three times with a gap of 7 days. Good \result has obtained an analysis.

III. RESULTS

III.I Solid state fermentation of the *Fusarium Moniliforme*- After 7 days of incubation pinkish white cottony colony was observed over the medium substrate. Fusarium fungi used up the medium substrate and the mycelium of the fungi spread over the plate.

Fig. 1 Solid state fermentation Plate of *Fusarium moniliforme 1100*

III.II HPLC of the extracted CURDE GA3 in Butanol with 0.04% GA3 powder as standard

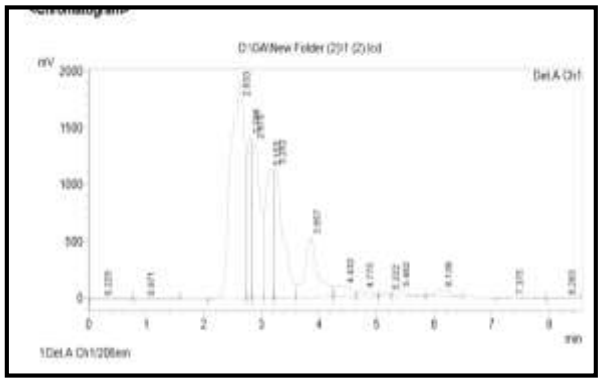

Fig. 2. HPLC chromatograms of GA3 of the crude extracted GA3

NO.	Sample	Peak time period	Peak Area	Purity%
1	GA3 STD.	2.7	1879559	93%
2	BL GA3 curde	2.699	25516258	5%

III.III Plant Experiment Results- The plant experiments were done in doubles using chickpea, Green gram seeds. The spraying schedule fixed was spraying after one week of the interval, on growing plants also data collection of the plants was done after subsequent spraying to analyze the effect of GA3. The results obtained were tabulated in form of germination percentage and weight gain. The table shows the best Average Height gain by chickpea and Green gram plants The reading of chickpea 16-day old plant **(**after two spraying processes at an interval of 8 days**).**

Fig.3. Green gram Plant pot experiment

Fig.4 Chickpea Plant pot experiment

III.III.I Plant experiment data of chickpea and green gram -The reading of chickpea 16-day old plant after three spraying processes at an interval of 21days

Formulations	No. of seeds germination	Average height	Total Branches	Total Leafs	No. of seeds germination	Average height	Total Branches	Total Leafs
NC	12	20.65	168	1388	25	9.3	45	70
TEST (0.5%)	13	24.7	178	1960	26	10.2	52	83
PC	14	22.5	103	1654	30	9.5	50	54

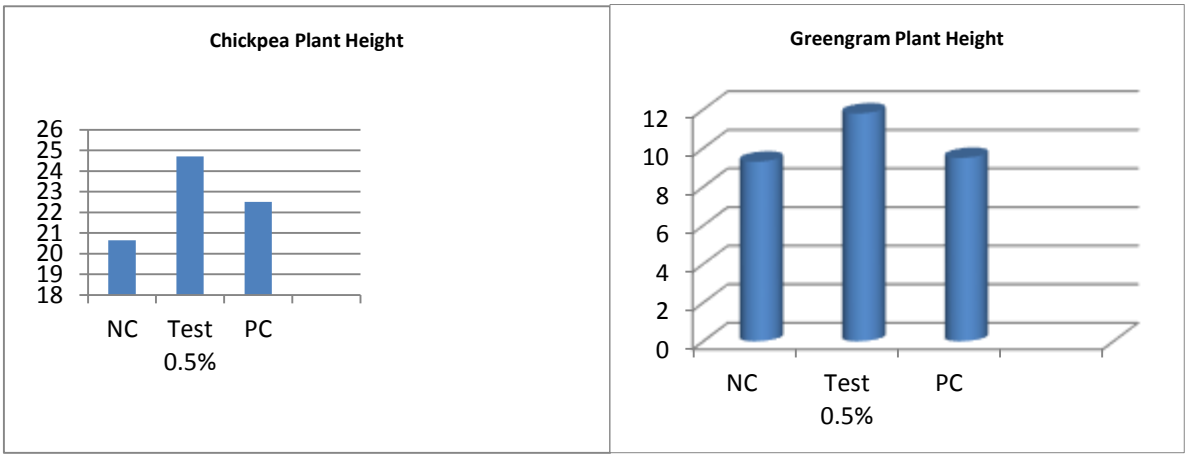

Fig.45: Chickpea Plant and Greengram Height data

III.III.II Plant growth observation - After 21 days of seed cultivation in pots, chickpea experimental plants which were treated with Butanol extract of *Fusarium moniliforme 1100* showed good branching of shoots but they were comparatively showing better height gain then plants of positive control but the negative control plants did not show proper branching and had poor weight gain then experimental plants.

IV. DISCUSSION

Gibberellic acid is a plant growth Hormone that comes from a fungus of the genus *Gibberella fujikuroi* the effects of Gibberellic acid on plants is observed as stem along action and good plant reproduction and growth. Currently, Gibberellic acid is largely produced by solid-state fermentation of Gibberella fujikuroi on the large industrial scale. The HPLC data interpreted the purity of Gibberellic acid to be 5%.which was found to be economically viable and of high commercial importance and the secondary metabolite GA3 plant PGPR was then used on experimental pot trails for confirmation of its effect on plant growth and quantification of increased product yield. The plant experiments confirm that GA3 can be used as PGPR which can be used in agriculture for getting better yield and high production from crops.

CONCLUSION

The study showed that the Solid state fermentation technique leads to a better production of secondary metabolite Gibberellic acid from the fungi *Fusarium Moniliforme 1100*. The Total yield of the Gibberellic acid obtained was 5%.which was cost effective and of high commercial importance, the experiential novel citric pulp substrate showed better growth and high product yield. The experiment was successful as it provides for an improved and cost-effective process for the higher production of Gibberellic acid from fungi.

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