Research Article **ISSN: 2349 – 7114**

Asian Journal of Research in Pharmaceutical Sciences and Biotechnology

Journal home page: www.ajrpsb.com

PRODUCTION OF GIBBERELLIN FROM *PSEUDOMONAS FLUORESCENS* AND *FUSARIUM OXYSPORUM* AND ITS GROWTH PROMOTING ACTIVITY ON VEGETABLE CROPS

A. S. Swarnalakshmi*[1], V. Manikandan[2], V. Veeramanikandan[3]

[1*]Department of Microbiology, Vivekanandha Arts and Science College for Women, Veerachipalayam, Sankari West Post, Salem District, Tamilnadu, India.

[2]Department of Microbiology, Kamadhenu College of Arts and Science, Dharmapuri, Tamilnadu, India.

[3]Department of Microbiology, M.G.R College of Arts and Science, Hosur, Tamilnadu, India.

ABSTRACT

Gibberellins are phytohormones that are produced within the plants and also by some microorganisms. They play an important role in seed germination, stem elongation, flowering, enzyme induction etc. The main objective of the present study is to isolate and identify the production of gibberellins from *Pseudomonas fluorescens* and *Fusarium oxysporum* and its effect on the growth promoting activity of plants. Soil sample and spoiled corn sample were selected for the isolation of *P.fluorescens* and *F.oxysporum*. The isolated microorganisms were identified by primary and secondary screening methods. They were mass cultivated in a semi-automatic fermentor and after fermentation the broth were extracted and filtered by membrane filtration method. The extracted gibberellin were estimated by colorimetric method using Zinc acetate reagent and further confirmed by HPLC analysis. The peak values were found to be 2.05, 2.40, 2.65 and 3.08 for *Pseudomonas* extract and for *Fusarium* extract, the peak values were found to be 1.47, 1.65, 1.83 and 2.86 respectively. The microbial extracts and the Standard Gibberellin were applied to four types of plants such as Chili, Tomato, Ladies finger and Brinjal. Both the extracts showed maximum growth promoting activity of 110 cm and 78 cm in tomato plants respectively. Unfortunately Brinjal showed no growth promoting activity by applying *Fusarium* extracts. From the results obtained, it can be concluded that the microbial extracts produce considerable quantities of Gibberellins.

KEYWORDS

Gibberellins, *Pseudomonas fluorescens*, *Fusarium oxysporum*, HPLC, Growth promoting activity.

Author for Correspondence:
Swarnalakshmi A S,
Department of Microbiology,
Vivekanandha Arts and Science College for Women,
Veerachipalayam, Salem, Tamilnadu, India.

Email: swarnasundar95@gmail.com

INTRODUCTION

Plant hormones are chemicals that regulate plant growth. Plant hormones are signal molecules produced within the plant, and occur in extremely low concentrations. Gibberellins are a type of microbial phytohormone having tetra carbocyclic diterpernes that regulate and influence the growth, developmental process (Zhang *et al*, 2007)[1],

flowering (Cleland and Zeevaart 1970)[2], stem elongation (Suge and Rappaport 1968)[3], seed germination (Ogawa *et al*, 2003)[4] and enzyme induction (Rogers and Rogers 1999)[5]. They were first discovered when Japanese researchers, including Eiichi Kurosawa, noticed a chemical produced by a fungus called *Gibberella fujikuroi* that produced abnormal growth in rice plants.

It has been reported that *Pseudomonas* sp. and *Streptomyces* sp. are bacteria that produce gibberellin molecule and gibberellin-like substances. Species of *Alternaria, Aspergillus, Cephalosporium, Cladosporium, Cylindrocarpon, Fusarium, Mycelium, Neurospora, Penicillium, Rhizopus, Sphaceloma, Trichoderma* and *Verticillium* are fungi known to produce gibberellins or gibberellin-like substances (Frankenberger and Arshad 1995)[6]. *R. japonicum, R. leguminosarum* and *R. Meliloti* are known to produce GA3 and GA9. *Pseudomonas* isolates from the rhizosphere of *Pyrus* and *Malus* produced gibberellins upto 25-60µg/ml. (Ritika Kapoor *et al*, 2016)[7]. On solid state fermentation, 7.5-fold and 3.5-fold increase in gibberellic acid production was observed upon addition of soluble starch and urea respectively, in CWB using *Fusarium moniliforme* (Panchal and Desai, 2016)[8].

The total yield of the Gibberellic acid was found to be about 5%which was cost effective and of high commercial importance. Better growth and high production yield was observed while using citric pulp as a substrate. (Rashmi Pal *et al*, 2017)[9].

MATERIAL AND METHODS

Sample collection

The soil samples were collected for the isolation of bacterial species around Dharmapuri district and the spoiled corn sample used for the isolation of fungi.

Primary Screening

The collected samples were subjected to crowded plate method for the primary screening of the microorganisms. This is done by serially diluting the sample and spread on the surface of the agar medium. The selected colonies were subcultured and purified by Streak plate method.

Secondary screening

This is done to detect the industrially important microorganisms. The isolates were screened by microscopic (Gram staining for bacteria and LPCB staining for fungi) and macroscopic (Kings medium for *Pseudomonas* and SDA medium for *Fusarium* species) identification methods.

Mass cultivation

The purified culture transferred to the liquid media for the large cultivation of microbes. 10 litres of the media (Nutrient broth for bacteria and Sabouraud Dextrose broth for fungi) were prepared and transferred to the semi-automatic fermentor, sterilized followed by the inoculation of the culture. They are incubated for seven days for the mass cultivation of the bacteria and fungi provided the optimum temperature, pH, aeration and agitation.

Preparation of the extract

The fully grown mycelia (after 4 days of incubation) were harvested and filtered for the separation of mycelia from the broth. The fully developed bacterial culture were collected and centrifuged and the supernatant were used for the further analysis. Filtration is done by membrane filtration method to remove microbes from the media (Tankeshwar, 2010)[10].

Estimation of Gibberellins

Gibberellins were estimated colorimetrically by the method (Holbrook *et al*, 1961)[11] of 2ml of zinc acetate reagent (21.9 g zinc acetate + 1ml of glacial acetic acid in 100ml of distilled water) mixed with 15ml of supernatant, allowed to room temperature for two minutes and add 2ml of potassium ferrocyanide (10.6% in distilled water). The components were centrifuged at 2000 rpm for 15 minutes. 5ml of 30% HCl taken in the test tube and added with 5ml supernatant and mixed component was incubated at 20°C for 75 min. The absorbance was read in a colorimeter and compared with the standard gibberellin.

HPLC analysis

Gibberellins was identified by HPLC at 254nm (Tien *et al*, 1979)[12] and sample was injected into HPLC at a wave length of 206 nm which was decided on the basis of detection of gibberellins by absorption (Barendse and Van De Werken, 1980)[13]. 20µl of sample was injected into the HPLC system.

The overall run time was 7.0 min and the flow rate was 1.0ml/min. Results were acquired and processed by internal software (Empower, Waters, Milford, MA, USA).

Field application of enzyme

The cultivated cultures were filtered by using membrane filter and used for field application. The filtered component were sprayed to various plants like Tomato (*Solanum lycopersicum*), Chili (*Capsicum annuum*), Brinjal (*Solanum melongena*) and Ladies Finger (*Abelmoschus esculentus*). Seeds were collected from local shop in Syngenta brand and cultivated in plastic pots. These plants were widely cropped in the Dharmapuri and Krishnagiri district. Each plant were divided into 4 groups; group 1 treated by *Pseudomonas* sample, group 2 treated by *Fusarium* sample, group 3 treated by standard gibberellins and finally the group 4 left as control without addition of anything. This treatment proceeded for 20 days once up to yielding.

RESULTS AND DISCUSSION

The primary isolation of the microbes were done by pure culture techniques. The confirmatory screening were done by microscopy and selective isolation method. Those were confirmed by the colony morphology.

For the production of gibberellins, the organisms were mass cultivated using fermentation method. The bacteria were mass cultivated in Nutrient broth and for the mass cultivation of fungi, Sabouraud dextrose broth were used. Extracts were taken from the mass culture.

HPLC analysis

Shwetha Sharma *et al*, (2012)[14] reported the production of gibberellins by *Pseudomonas fluorescens* isolated from the rhizospheric soil of apple and pear of Himachal Pradesh. Gibberellin production was found to be 116.1 - 485.8µg/ml. Two isolates having peak values of 894 - 1010 were observed.

In this process, *Pseudomonas sp.,* sample were analysed in the high performance liquid chromatography technique and got peak level in 2.05, 2.40, 2.65 and 3.03. *Fusarium sp,* sample produced peak in 1.47, 1.65, 1.83, 2.86 and etc.

Field application

The microbial extract and standard gibberellins were applied to the plants for the identification of growth promoting activity of various plants. Four types of plants were used for identification process namely, Chili, Tomato, Brinjal and Ladies finger.

In chili plants, standard gibberellins showed better growth and gives better yield compared to the control plants. Leaves and branches seems healthy and fresh without any diseases in the test and standard batch of the plants. The control plants were affected by bacterial leaf diseases and fungal leaf diseases but not in high level. *Fusarium* extract induced plant growth minimum in 40 cm and maximum 53cm in height, *Pseudomonas* extract induced the growth of plant minimum in 42cm and maximum in 51cm. The standard gibberellins gave growth in the range of minimum 45cm and maximum 58cm height of the plants. But the control plants were grown in 28cm of minimum height and 35cm of maximum height. These results were indicates that, *Fusarium* and *Pseudomonas* extracts induce the plants growth.

In tomato plants, *Fusarium* and *Pseudomonas* extracts produced better results compared to control plants but not more than standard gibberellins applied plants. Tomato plants were grown in 60 to 78cm height and 18 to 25 fruits per plant in *Fusarium* applied plants. On the other hand, 75cm to 110cm of height and 22 to 26 fruits per plant in *Pseudomonas* extract applied plants and the same results came in the standard gibberellins applied plants. It indicates that, the activity of *Pseudomonas* extract gave better yielding in the tomato plants. But the control plants did not grow more than 60cm of height and 13 to 15 fruits were produced per plant.

In ladies finger plants, standard gibberellins gave good growth and of plants and induce the yield. It induce the plants growth between 64cm to 67cm of height and gives 18 to 20 fruits per plants. In *Fusarium* extract applied plants, 56cm to 62cm of height and 12 to 14 fruits were collected. At the same time *Pseudomonas* extract applied plants grow in 85cm to 92cm of height and gives 10 to 13 fruits per plants. *Pseudomonas* extract which gives better growth but not in yield when it compared to

Fusarium and standard gibberellins applied plants. In the control plants, plants were grown up to 45cm of height and gives 8 to 10 fruits per plants.

Unfortunately, in brinjal plants, both *Fusarium* extract and *Pseudomonas* extract did not gave better result compared to control plants. The control plants were grown up to 55cm in height and gives 10 to 12 fruits per plant. At the same time the standard gibberellins applied plants were grow up to 58cm to 61cm of height and gives 10 to 13 fruits per plants. But the both testing plants grow not more than 49 cm of height and gives 6 to 8 fruits per plant. Even the number of branches are lower than the control and standard plants. Both *Fusarium* and *Pseudomonas* extract were did not act well in the testing plants.

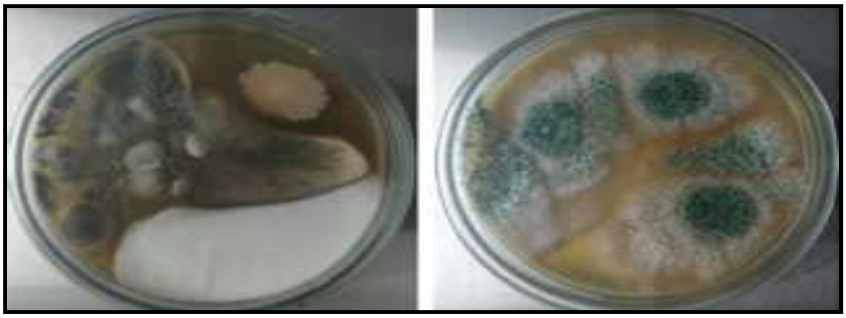


Figure No.1: Primary and secondary isolation of *Fusarium* sp.

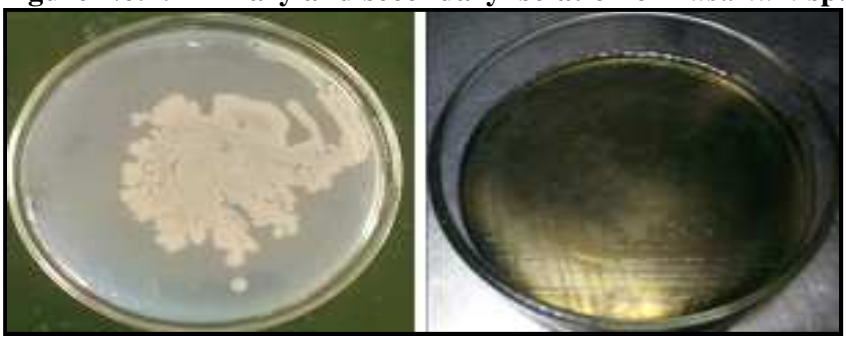
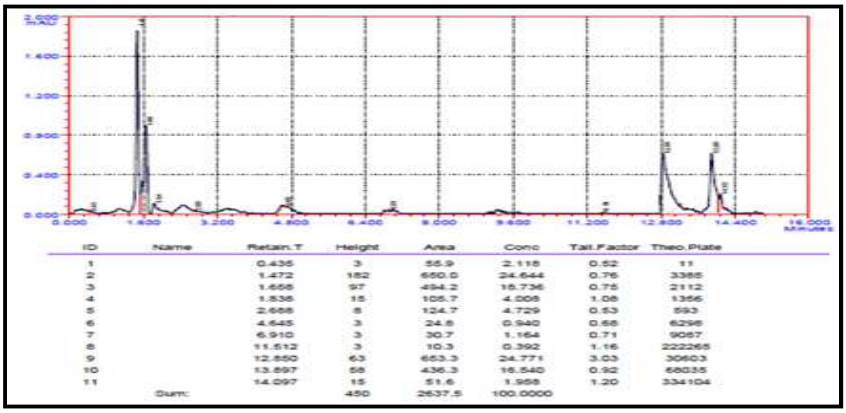


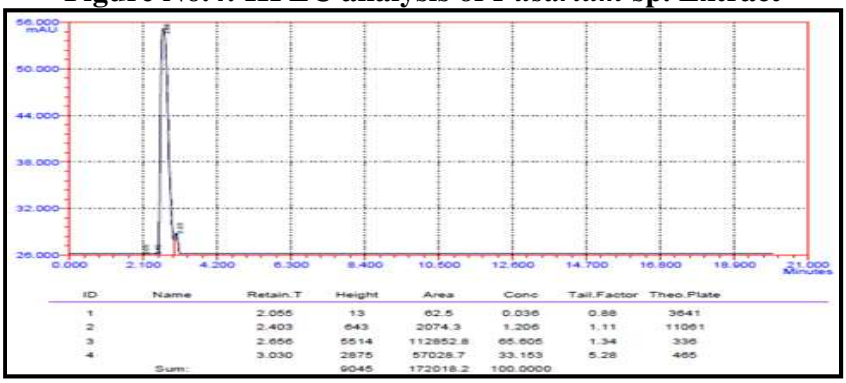
Figure No.2: Primary and secondary isolation of *Pseudomonas* sp.

Figure No.3: Extraction of *Fusarium* sp., (F) and *Pseudomonas* sp. (P)



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		0.435	3	55.9	2.118	0.52	11
2		1.472	182	650.0	24.644	0.76	3385
3		1.658	97	494.2	15.736	0.75	2112
4		1.838	15	105.7	4.006	1.06	1356
5		2.668	8	124.7	4.729	0.53	593
6		4.645	3	24.6	0.940	0.68	6296
7		6.910	3	30.7	1.164	0.71	9067
8		11.512	3	10.3	0.392	1.16	222265
9		12.850	63	653.3	24.771	3.03	30603
10		13.697	59	436.3	16.540	0.92	68035
11		14.097	15	51.6	1.958	1.20	334104
	Sum:		450	2637.5	100.0000		

Figure No.4: HPLC analysis of *Fusarium* sp. Extract



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		2.056	13	62.5	0.036	0.88	3641
2		2.403	643	2074.3	1.206	1.11	11061
3		2.656	5514	112852.8	65.605	1.34	336
4		3.030	2875	57028.7	33.153	5.28	465
	Sum:		9045	172018.2	100.0000		

Figure No.5: HPLC analysis of *Pseudomonas sp.* extract

Analysis of plant Growth

Figure No.6: Initial stage of the plants

Figure No.7: Yielding stage of Ladies Finger plant

Figure No.8: Yielding stage of Tomato plants

Figure No.9: Yielding stage of Chili plants

Figure No.10: Yielding stage of Brinjal plants

CONCLUSION

The present study showed that the microbial extracts have the ability to produce considerable quantity of gibberellins. From this study, it can be concluded that microbial extracts of gibberellins also shows good plant growth promoting activity and yield. The microbial extracts gave results more or less nearby standard gibberellins.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Bioplant Tech Research and Development Laboratory, Dharmapuri, Tamilnadu, India for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

Due to over population and the need of high productivity of crops in lesser time made the people

to use chemical fertilizers which pose a threat to the environment as well as the human lives. In order to overcome such problems, this research work was carried with a vision to enhance the quality and quantity of crops in an eco-friendly manner.

BIBLIOGRAPHY

1. Zhang J, Ren H, Gao Z, Chen L, Wei K, Liu J, Fan Y, Davies W J, Jia W. "Dynamic analysis of ABA accumulation in relation to the rate of ABA catabolism in maize tissues under water deficit", *Journal of Experimental Botany,* 58(2), 2007, 211-219.
2. Cleland C F, Zeevaart J, A D. Gibbetellins in relation to flowering and stem elongation in the long day plant, *Plant Physiology,* 46(3), 1970, 392-400.
3. Suge H and Rappaport L. Roll of Gibberellins in stem elongation and flowering in radish, *Plant Physiology,* 43(8), 1968, 1208-1214.
4. Ogawa Mikihiro, Atsushi Hanada, Yukika Yamauchi, Ayuko Kuwahara, Yuji Kamiya, and Shinjiro Yamaguchi. Gibberellin biosynthesis and response during Arabidopsis seed germination, *Plant Science Center, RIKEN, The Plant Cell,* 15(7), 2003, 1591-1604.
5. Rogers S W and Rogers J C. Cloning and characteristics of a gibberellins induced RNase expressed plants, *Plant Physiology,* 119(4), 1999, 1457-1464.
6. Frankenberger W T and Arshad M. Effects of Streptomyces Biofertilizer to Soil Fertility and Rhizosphere's Functional Biodiversity of Agricultural Plants, *Phytormones in Soils, Marcel Dekker Inc, New York,* 1995, 35-71.
7. Ritika Kapoor, Ruchi Soni and Mohinder Kaur. "Gibberellins production by fluorescent *Pseudomonas* isolated from Rhizospheric soil of *Malus* and *Pyrus"*, *International Journal of Agriculture, Environment and Biotechnology, IJAEB,* 9(2), 2016, 193-199.
8. Panchal R R and Desai P V. "Study of gibberellic acid production by solid state fermentation using *Fusarium moniliforme* sheldon", *International Journal of Applied Sciences and Biotechnology,* 4(3), 2016, 402-407.
9. Rashmi Saxena Pal, Yogendra Pal, Pranay Wal. In-House Preparation and Standardization of Herbal Face Pack, *The Open Dermatology Journal,* 11(1), 2017, 72-80.
10. Tankeshwar. Microbiologist at Department of Microbiology and Immunology, *Patan Academy of Health Sciences, Nepal,* July 13, 2010.
11. Holbrook A A, Edge W L W and Bailey F. Spectrophotometric method for determination of gibberellic acid in gibberellins, *ACS Washington, D.C,* 18, 1961, 159-167.
12. Tien T, Gaskin M, Hubbell D. Plant growth substances produced by Azospirillum brasilense and their effect on the growth of pearl millet (Pennisetumamericanum L), *Applied Environmental Microbiology,* 37(5), 1979, 1016-1024.
13. Barendse G W M and Van De Werken P H. High performance liquid chromatography of gibberellins, *Journal of Chromatography,* 198(4), 1980, 449-455.
14. Shweta Sharma, Mohinder Kaur, and Durga Prashad. University of horticulture and forestry, Nauni, "Isolation of fluorescent pseudomonas strain from temperate zone of Himachal Pradesh and their evaluation as plant growth promoting rhizobacteria (pgpr)", The Bioscan, *An International Quarterly Journal of Life Sciences,* 9(1), 2014, 323-328.

Please cite this article in press as: Swarnalakshmi A S *et al.* Production of gibberellin from *pseudomonas fluorescens* and *fusarium oxysporum* and its growth promoting activity on vegetable crops, *Asian Journal of Research in Pharmaceutical Sciences and Biotechnology,* 7(3), 2019, 53-59.