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ORIGINAL ARTICLE

In Vitro Production of Gibrellic Acid from *Trichoderma Harzianum*

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ABSTRACT
Trichoderma harzianum is well known for its bio control activities, but recent studies also shows that it promotes plant growth and also influence various physiological growth promoting activities in plants. Therefore investigation was carried out to study possible ways of plant growth promotion by Trichoderma harzianum. Trichoderma harzianum was isolated from the soil samples of Sanganer District and fermentation was carried out in three liquid culture media viz. Borrow, Darken and Stodola. Trichoderma harzianum produces extracellular gibberellic acid. Highest gibberellic acid was produced in Borrow followed by Darken and Stodola culture media.
Keywords: *Trichoderma, Fermentation, Gibberellic acid*

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INTRODUCTION

Trichoderma spp. is free-living fungi that are common in soil and root ecosystems. It has gained immense importance since last few decades due to its biological control ability against several plant pathogens. *Trichoderma* species are being employed widely in plant agriculture, both for disease control and increase yield [2, 1]. In addition to the ability of *Trichoderma* spp. to attack or inhibit the growth of plant pathogens directly, recent discoveries indicate that *Trichoderma* spp. have evolved in multiple mechanisms, that result in improvements in plant resistance to disease and plant growth and productivity [3, 7].These new findings are drastically changing our knowledge of the mechanisms of action and uses of this fungus.

Recently, many workers observed that *Trichoderma* spp. have diverse antifungal mechanisms and ability to promote plant growth. It stimulate plant growth in cucumber, cabbage, lettuce, potato, tomato, carrot, beans and peas [5, 6, 8, 9]. Presumed mechanisms involved in the stimulation of plant growth by *Trichoderma* include interactions with plant roots similar to Mycorrhizae, in which *Trichoderma* penetrates and colonizes root tissues without eliciting specific defense responses against the colonizing strain [10]. Other possible explanations of this phenomenon include, control of minor pathogens leading to stronger root growth and nutrient uptake [5], secretion of plant growth regulatory factors such as phytohormones [1] and release of soil nutrients and minerals by increased saprophytic activity of *Trichoderma* in the soil [5].This study was undertaken with the objectives to find out possible cause behind plant growth promotion by *Trichoderma* spp.

MATERIALS AND METHODS

Isolation and identification of *Trichoderma* strains:

Different strains of *Trichoderma* spp were isolated from soil samples of Sangamner district by serial soil dilution technique. 1ml of 10^6 dilution of each soil solution was poured on Rose Bengal Agar plate and incubated at 28 °C for one week. The culture plates were examined daily and each colony was considered to be one colony forming unit (cfu). After enumeration of cfu, individual colonies were isolated from the same plates and each uncommon colony transfer on a fresh Potato Dextrose Agar (PDA) plate. Distinct

morphological characteristics were observed for identification, and the plates were stored at 4 °C. Two techniques, visual observation on petri dishes and micro-morphological studies on slide culture, were adopted for identification of *Trichoderma harzianum* species. For visual observation, the isolates were grown on PDA medium for 3-5 days. For micro-morphological studies, a slide culture technique was used. Examination of the shape, size, arrangement and development of conidiophores or phialides provided a tentative identification of *Trichoderma harzianum.* [4].

Maintenance of pure culture of isolated organism:

Colony was purified by transfer and retransfer on fresh PDA plates, pure culture will be maintained on PDA slants and slants were maintained in incubator at 28^0C.

In vitro production of gibbrellic acid:

Subculture of the isolated pure strain of *Trichoderma harzianum* was done at 28°C on potato-dextrose agar plate and incubated for six days. Inoculum disc of 10 mm was added in sterile 50 ml nutrient medium flasks. (Ammonium chloride 3.5g;Sucrose 30g Ammonium sulphate 2 g, Calcium carbonate 7g; Distilled water 1000 ml.) The inoculated flasks were incubated at 28°C for 48 hrs. on a mechanical agitator. This inoculum was used at a concentration of 1.5% for each flasks of three different fermentation media viz; Borrow (Dextrose-40 g; Ammonium tartrate 9.5 g ; Mono potassium phosphate 2.0 g ;Potassium sulphate 0.2g and Distilled water1000ml), Darken (Corn steep liquor-25g, Dextrose 20g ; Ammmonium sulphate 1g; Mono potassium phosphate 0.5g ; Distilled water 1000ml.) and Stodola medium (Dextros 20 g ; Ammonium chloride 3g; Mono potassium phosphate 3.0g; magnesium sulphate 3.0 g; Distilled water 100ml). Fermentation was carried out in 100 ml conical flask containing 50 ml liquid medium, for 192 hours. at 28°C . After192 hours the broth was filtered with whatman filter paper No. 4 these filtrates were used for HPLC analysis

Determination and estimation of gibberellic acid:

Gibberelliic acid content was estimated by HPLC, standard cure was prepared by standardization of pure gibberllic acid. The chromatographic conditions were established and kept constant throughout the experiment. Detection wavelength was kept on 254 nm; 20 µl sample was used for injection.

RESULTS AND DISCUSSION

Trichoderma harzianum was successfully isolated from soil and fermentation was carried out on three different broths as Borrow, Darken and Stodola. Fermented broth were analysis for their properties like color change from pale yellow to golden yellow, fishy odor and pH change 7 to 5.5. Indicates that *Trichoderma harzianum* produce some extracellular chemicals which results in change in the pH of broth. 20 ul broth samples were tested for gibberellic acid content. *Trichoderma herzianum* produced gibberllic acid in all media. Comparatively highest gibberellic acid production was found in Borrow (36.03 µg/ml) followed by Darken (2.25 µg/ml) and Stodola medium (1.76µg/ml).

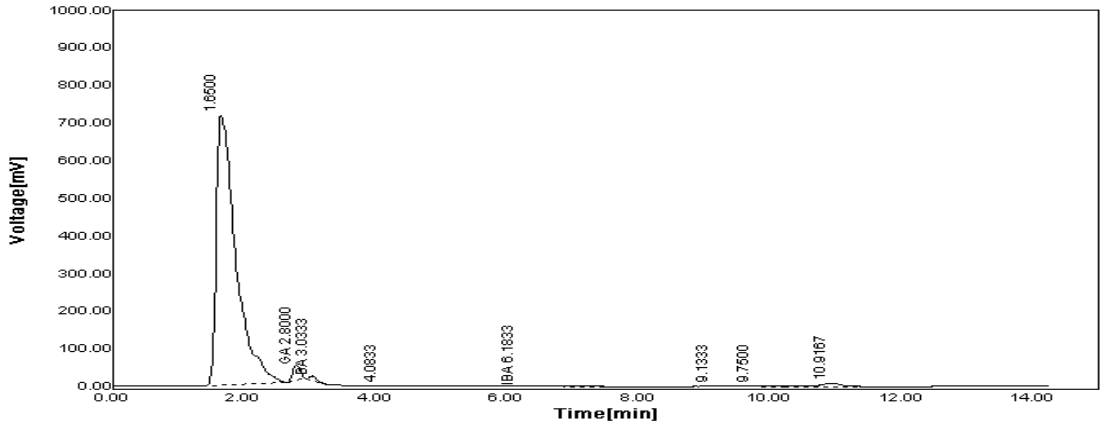

Fig1: HPLC chromatogram of Fermented Broth

DISCUSSION

Fermentation of *Trichderma harzianum* in liquid media produces extra cellular plant growth regulators leads to change in colour pH and odor. HPLC detection supported Borrow medium as proper medium for gibbrellic acid production.

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