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BIOPRODUCTION OF INDOLE 3-ACETIC ACID BY *TRICHODERMA* STRAINS ISOLATED FROM AGRICULTURE FIELD SOILS IN SENEGAL

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ABSTRACT

A total of twenty *Trichoderma* strains were isolated from rhizospheric soils of tomato fields from different areas of Niaye zone, main area of horticulture production in Senegal. Identification of these *Trichoderma* isolates was based on morphological and cultural characters on potato dextrose agar medium. Of the 20 strains, only 8 strains showed the maximum indole acetic acid production. Among the 8 strains TG 4 showed maximum (90 µg/ml) indole acetic acid production was selected for optimization studies. Optimum conditions for maximum IAA were 8 days of incubation and pH 7.0 at 37°C temperature. Among the carbon and nitrogen sources tested, Mannitol and yeast extract induced the maximum IAA production of 105µg/ml and 95µg/ml respectively. Possession of plant growth promoting traits like *Trichoderma* TG 4 strain a promising strain to be developed as multifunctional biofertilizer.

KEYWORDS: *Trichoderma*, Indole acetic acid (IAA), Potato dextrose agar (PDA).

1. INTRODUCTION

Rhizosphere is a wealthy location of microbes, ecological niche and should be explored for potential plant growth promoting rhizobacteria (PGPR), which developing as bio- inoculants for interact with plant roots and enhancement of yield of crop plants. There were several plant growth promoting rhizobacterial (PGPR) inoculants that seems to promote plant growth through different mechanism such as plant growth hormone production, nutrient acquisition and plant disease suppression. Indole acetic acid (IAA) is one of the major physiologically active auxins, a product of L-tryptophan metabolism produced by several microorganisms including Plant Growth Promoting Rhizobacteria (PGPR) (Lynch, 1985). Different soil microorganisms including bacteria (Stein et al., 1990), fungi (Finnie and Van Staden, 1985) and algae (Rifat Hayat et al., 2010) are capable of producing physiologically active quantities of auxins, which may exert prominent effects on plant growth and development. The application of single and combined application of microbes could increase plant growth of cotton due to result of slightly deleterious effect of strain causing increased root leakage/damage, which allows a greater population of aggressive rhizosphere and root colonizers such as *Trichoderma viride* and *Pseudomonas fluorescence* (Shanmugaiah et al., 2008).

2. MATERIALS AND METHODS

i). Isolation of fungi

The rhizosphere soil samples from the tomato fields of 5 different areas of Niaye zone, main area of horticulture production in Senegal: UCAD, Sangalkam, Gorome, Notto Gouye Diama and Mboro were collected for the study. *Trichoderma* spp. were isolated on Potato Dextrose Agar (PDA) medium by soil dilution plate technique (Rapilly, 1968) using 10^{-3} to 10^{-5} dilutions. The plates were incubated at 28 ± 2°C for 5 days. *Trichoderma* colonies appeared in the plates were noted and sub cultured. After purified by single spore isolation method and they were maintained on potato dextrose agar (PDA) slants. Identification of *Trichoderma* isolates was based on culture characters as well as microscopic parameters (conidiophores branching, phialides shape and position, spore size and shape) (Nagamani *et al.*, 2006). The pure cultures were stored in the refrigerator at 4°C for further studies.

ii). Screening of isolates for IAA production

IAA production was determined based on the method described by Patten and Glick (2002) with slight modifications. Spores (100 µl) of *Trichoderma* sp were inoculated in nutrient

broth supplemented with 0.2% filter sterilized (0.2µm membrane filter, Whatmann) L-tryptophan solution and incubated at 28°C in a rotary shaker at 120 rpm for 7 days. After 7-days of incubation, the culture was centrifuged at 10000 rpm for 15 min. One milliliter of supernatant was mixed with 2 ml of Salkowski reagent (1ml of 0.5M $FeCl_3$ in 50mL of 35% $HClO_4$) and incubated for 1hr. Development of pink colour indicated the production of IAA. The quantification of IAA was read at 530 nm in a UV- Vis spectrophotometer. A standard curve was plotted for quantification of IAA solution and uninoculated medium with a reagent as a control. The amount of IAA in the culture was expressed as µg/ml (Gordon and Weber, 1951).

iii). Optimization of parameters for IAA production by *Trichoderma* TG4

The production of IAA was optimized for selected isolate *Trichoderma* TG4 by one factor at a time was employed in this study.

iv). Effect of L-tryptophan concentration

The effect of L-tryptophan concentrations on IAA production was studied using production medium supplemented with L-tryptophan at concentrations of 50, 100, 150, 200, 250, 300, 350 and 400 mg/100ml at pH 7.0. The culture was incubated at 37°C in a shaker at 200 rpm for 7 days and production of IAA was measured.

v). Effect of incubation time

The effect of incubation time for IAA production by *Trichoderma* strain TG4 was grown in 50 ml of production medium supplemented with 100 mg/ 100ml of L-tryptophan at pH 7.0 and incubated at 37°C in a shaker at 120 rpm for 7 days. IAA production was assayed by incubating *Trichoderma* culture under optimum conditions up to 8 days. Production of IAA and residual L- tryptophan was measured at every 24 h interval.

vi). Effect of pH

The optimum pH for the production of IAA by *Trichoderma* strain TG4 was determined with different pH value 2, 3, 4, 5, 6, 7, 8, 9 and 10. The culture was incubated at 37°C in a shaker at 200 rpm for 7 days. After incubation production of IAA was measured.

vii). Effect of carbon sources

In the production medium, mannitol was replaced with 5 different carbon sources (Arabinose, glucose, lactose, xylose, fructose, sucrose and maltose,) at 1% concentration inoculated with

Trichoderma strain TG4 and incubated for 8 days on rotatary shaker at 200 rpm at room temperature. Control was maintained without carbon source. IAA production was measured after incubation by using spectrophotometer at 540 nm.

viii). Effect of nitrogen sources

In the production medium, Yeast extract was replaced with different Nitrogen sources (Ammonium sulphate, Ammonium nitrate, Potassium chloride, L-Asperagine, L- glycine, Peptone and Beef extract) at 0.5 % level along with L-Tryptophan. IAA production was measured by using spectrophotometer at 540 nm.

3. RESULTS AND DISCUSSION

i). IAA production by *Trichoderma* strains

Indole Acetic Acid production was observed in 8 *Trichoderma* strains out of 20 strains tested. Much variation in growth and IAA production was observed in all the 8 positive *Trichoderma* strains (Fig.1). The strain TG4 showed highest IAA production of 90 µg/ml. More than 50 µg/ml of IAA production was observed in the remaining strains. The lowest IAA production was observed with the *Trichoderma* strain TM1. Similarly, IAA production was also reported by Gravel et *al*., (2007) and (Wesam) et *al*., 2017 using different species of *Trichoderma*.

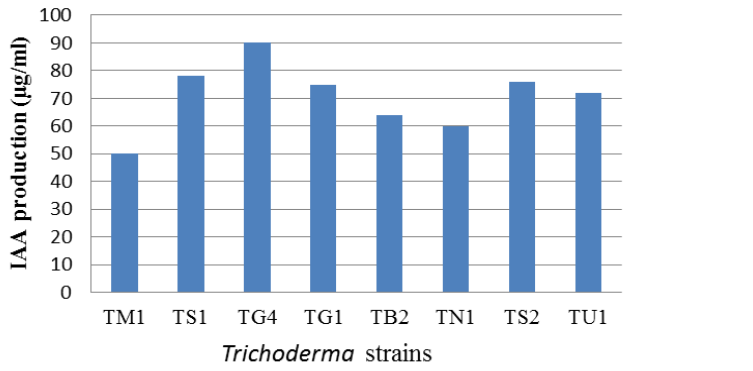

Fig. 1 Indole acetic acid production by *Trichoderma* strains after 72h incubation.

ii). Effect of incubation period on IAA production

The IAA production was observed between 4 to 11 days of incubation (Table-2). The maximum IAA production was observed on 8th day of incubation (90µg/ml). A progressive increase in production of IAA production by TG4 *Trichoderma* strain was observed up to 8 days of incubation and then a reduction in IAA production was recorded from 9th day onwards. However, Wasim et al (2017) reported that *T. harzianum* WKY1 produced 138.9 µg/ml of IAA after 5 days of incubation.

Reduction of IAA production at the later might be due to release of IAA degrading enzymes by the bacteria (Hunter, 1989).

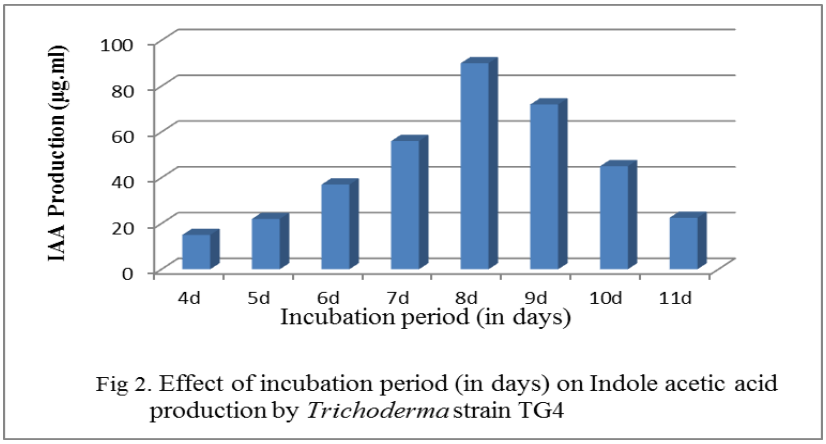

Fig 2. Effect of incubation period (in days) on Indole acetic acid production by *Trichoderma* strain TG4

Effect of L-Tryptophan concentrations on IAA production

Different concentrations of L-tryptophan between 50 to 400 mg/100ml were tested to study their effect on IAA production (Table-3). A gradual increase in IAA production with the increase in L-tryptophan concentration up to 300mg/100ml was observed, with maximum IAA production of 220 µg/ml. Thereafter a reduction in IAA production was observed. Wasem et al (2017) reported that maximum IAA of 138.9 µg/ml was produced by *T. harzianum* WKY1 strain at 106mg/100ml concentration of L-tryptophane. This clearly shows that strain difference was there among *Trichoderma* species.

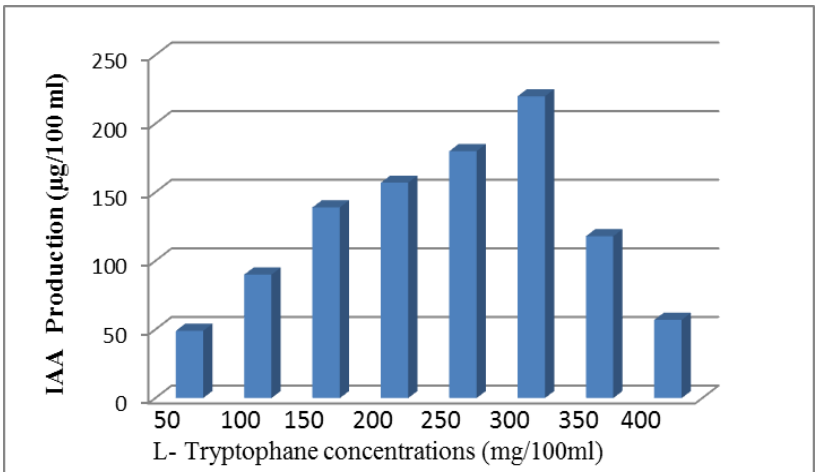

Fig 3. Effect of L-Tryptophane concentrations on IAA production by *Trichoderma* strain TG4

iii). Effect of pH on IAA production

The pH of the medium showed a significant influence on the production of IAA by *Trichoderma* strain TG4 (Fig. 4). The maximum IAA production (90 µg/ml**)** was observed at pH 7. A progressive increase in production of IAA by TG4 *Trichoderma* strain from pH 3 to 7 was observed and a reduction of IAA production was observed from pH 8 onwards.

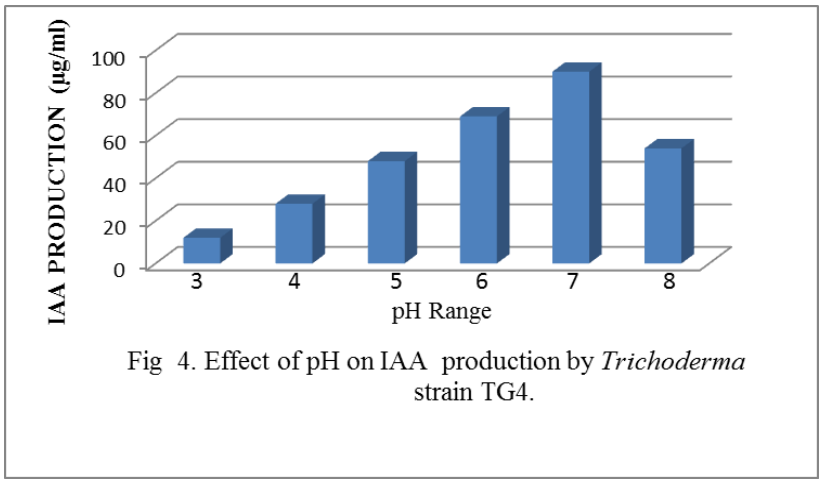

Fig 4. Effect of pH on IAA production by *Trichoderma* strain TG4.

iv). Effect of different temperature on IAA production

For effect of different temperature on IAA production by TG4 *Trichoderma* strains, our investigations showed that, the maximum IAA production was obtained at 30° (90 µg/ml) followed by 35° (58 µg/ml) and 40° (47 µg/ml) respectively. Similarly, IAA production was also high at the optimal temperature range of 25-30° (Hasan, 2002, Nathan et *al*., 2017).

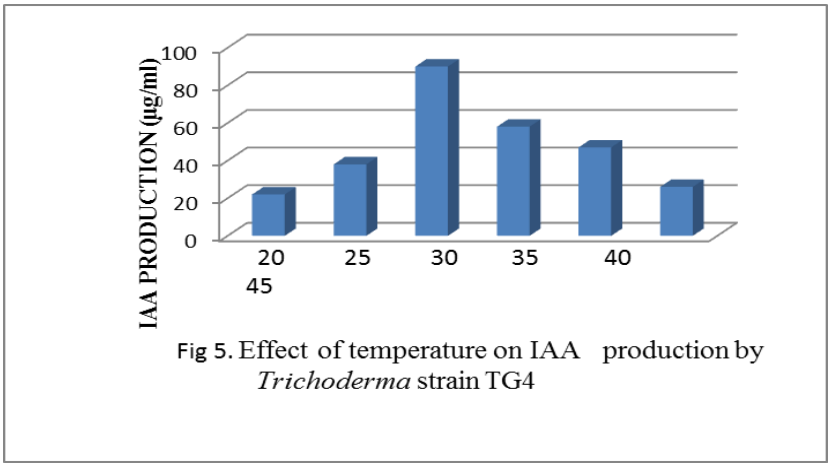

Fig 5. Effect of temperature on IAA production by *Trichoderma* strain TG4

v). Effect of different carbon source on IAA production

Eight different carbon sources (mannitol, glucose, sucrose, fructose, maltose, lactose, Arabinose, xylose) were studied for their effect on IAA production by *Trichoderma* strain

TG4 (Fig. 6). Mannitol in the medium gave maximum IAA production (105µg/ml) followed by sucrose (85µg/ml**)** and arabinose (81µg/ml). The lactose showed the lowest IAA production (54µg/ml) compared to the other carbon source. However Sucrose was repoted to supported maximum IAA production was reported by Wasem et al (2017) in *T. harzianum* WKY1.

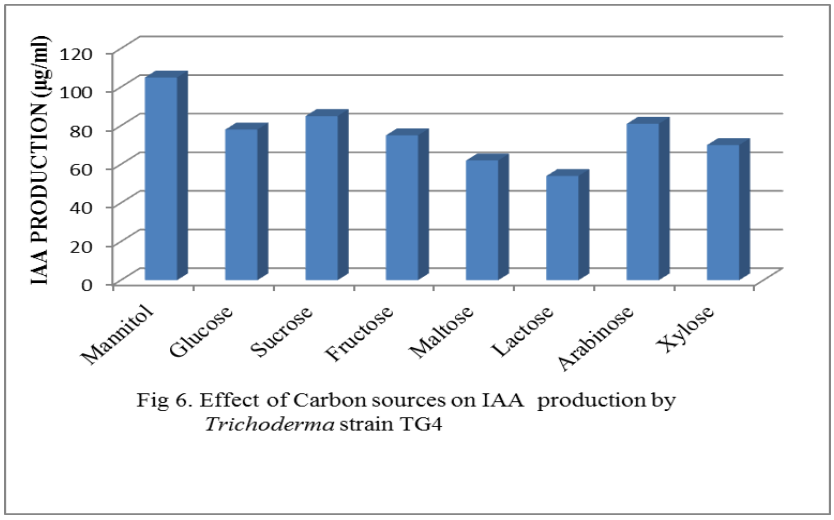

Fig 6. Effect of Carbon sources on IAA production by *Trichoderma* strain TG4

vi). Effect of different nitrogen sources on IAA production

Effect of nitrogen sources on IAA production by *Trichoderma* strain TG4 was studied by addition of various nitrogenous compounds (ammonium sulphate, potassium chloride, L-aspargine, Yeast extract, Ammonium nitrate, peptone, beef extract and L-glycine). These nitrogen source have a significant effect on IAA production (Fig. 7). Among all the nitrogen sources used, yeast extract was found to be the best nitrogen source for IAA production. Similar results was reported by Ton That Huu Dat et *al*., (2015) in *Bacillus subtilis.*

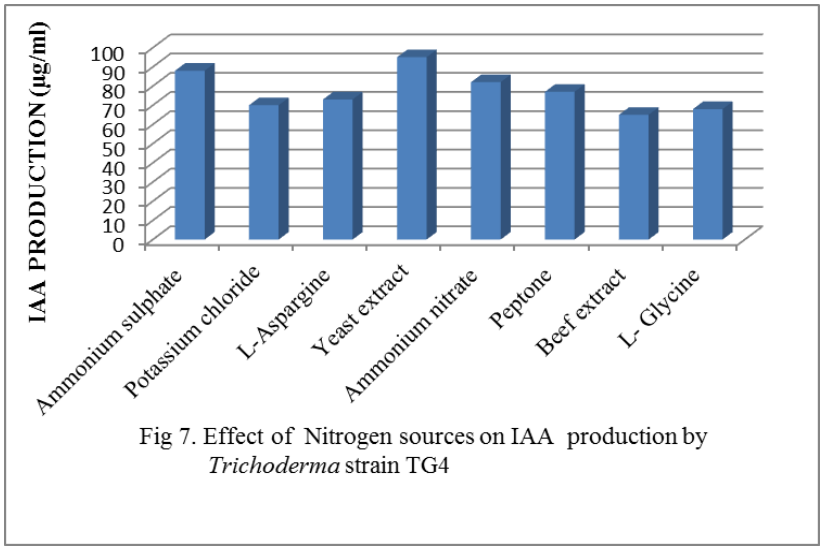

Fig 7. Effect of Nitrogen sources on IAA production by *Trichoderma* strain TG4

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