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Optimization and Characterization of Indole Acetic Acid Producing Efficiency of *Talaromyces trachyspermus* for Sustainable Agro-practices

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Abstract: The production of indole acetic acid (IAA) by plant growth-promoting microorganisms is one of the most important factors for plant growth. Present research work deals with the characterization and optimization of different physiological conditions for IAA production by the fungus *Talaromyces trachyspermus*. Different environmental factors and medium components were optimized for the production of IAA by fungal culture. Effect of pH, temperature, aeration and concentration of precursor i.e. L-Tryptophan was evaluated on the biomass of fungus along with the IAA production. This work also focused on the effect of different carbon and nitrogen sources in the media for the growth of *Talaromyces trachyspermus* and IAA production The maximum production of IAA was observed in 15 days of incubation under the condition of 6.5 pH, 28°C temperature, and 140 RPM, in presence of 0.5% of tryptophan, 4% glucose and 0.1% sodium nitrate. After optimization, the yield of IAA by fungal culture was increased up to 0.78 fold as compared to initial production. IAA production by fungal culture was confirmed by thin-layer chromatography and HPLC analysis. Optimization of IAA production by *Talaromyces trachyspermus* can be useful for the large-scale production of agriculturally important bioactive metabolites. Moreover, the plant growth-promoting efficiency of this fungal culture makes it novel bio-inoculants for sustainable agriculture.

Keywords: Indole Acetic Acid, Plant Growth-Promoting Fungi, *Talaromyces trachyspermus*, Optimization, Sustainable Agriculture

1. Introduction

With the increasing population, the demand for food and other agricultural resources is increasing simultaneously. Agricultural scientists have developed various chemical fertilizers to fill the deficit gap in the demand and production of agricultural products. But in the long term, it adversely affects the soil and misbalances the mineral proportion for flora and fauna of that local region. To solve this problem, new scientists are focusing to develop new biofertilizers to rejuvenate soil fertility along with surplus crop production.

Many biotic and abiotic factors of soil affect the growth and development of plants. The layer of soil that surrounds the roots have significant importance due to having immense metabolic activities of plant and microorganisms [1]. Various microbes such as bacteria, fungi, actinomycetes, protozoa and algae exist in this rhizospheric zone [2]. The establishment of beneficial microflora around the rhizospheric region of the plant depends on the type of organic compounds released by the roots of the host plant [3]. Moreover, this establishment of microorganisms influences other plant growth-promoting activities in and around the host plants. Plant growth-promoting fungi (PGPF) are a group of microorganisms that denotes diverse genera of nonpathogenic fungi that give different benefits to their host plants [2]. It has been reported that meeting the rising feed demands of the increasing population is the biggest challenge [4]. So the application of PGPF is the effective, economic

and environmentally safe agro-input to achieve the target of sustainable agriculture [5]. Many plant growth-promoting fungal genera *Trichoderma, Aspergillus, Phoma, Penicillium, Talaromyces, Chaetomium, Fusarium* and *Piriformospora* are well known reported PGPF [6]. These fungi have the natural ability to the promotion of plant growth in different crops [7–9]. Many studies showed that the inoculation of PGPF in different dicots and monocots induces better germination rate, seedling vigour, root-shoot growth, flowering, photosynthetic ability, soil health and crop yield [10]. Recent research reported that these fungal species show their plant growth-promoting potency through the production of various bioactive compounds such as phytohormones and volatile compounds [11, 12]. The ability of PGPF for plant growth promotion may also revel from enhanced nutrient availability, amelioration of abiotic stresses, and antagonism to phytopathogens [8, 13].

Indole-3-acetic acid (IAA) is an important phytohormone and member of the auxins family. It is an essential component for various plant metabolisms such as embryo development, abscission, phototropism, geotropism, fruit development etc. IAA helps to plant nutrient uptake from its rhizospheric surroundings by promoting the root length, root branches, root hairs and lateral roots [14]. Many plant growth-promoting microorganisms are reported for releasing IAA as their secondary metabolites by using the root exudates as a substrate [15, 16]. By the production of this important phytohormone, microbes can make a healthy ecological niche between the plant and microorganisms [15, 17].

The present study aimed to enhance the production of IAA by *Talaromyces trachyspermus* by optimizing the different environmental parameters. This fungal culture is known for many plant growth-promoting attributes [18] and can be further used as potent bioinoculants for many crops.

2. Material and Methods

2.1. Procuring of Fungal Culture

The fungal strain was screened from foliar tissues of the medicinal plant *Withania somnifera* collected from the different regions of Bhopal District M. P. India in 2016. The potent fungus, *Talaromyces trachyspermus* 4014 was confirmed by BLAST and phylogenetic analysis of sequences of rDNA ITS, LSU (D1 D2), and β-tubulin genes with accession number MF509777 [18]. The Culture was procured from the Department of Microbiology, Barkatullah University, Bhopal (M.P.), and inoculated on Potato Dextrose Agar (PDA) medium supplemented with streptomycin (100 mg/L) for seven days at 28°C. After the incubation of seven days, pure colonies of fungus were preserved in Potato Dextrose Agar slant at 4°C in the refrigerator.

2.2. Indole Acetic Acid Production by Talaromyces Trachyspermus

Indole acetic acid production through fungal culture was estimated by the standard method [19] with slight modification. The procedure was done using 20 ml Potato Dextrose Broth amended with 0.1% L tryptophan. 14 days old agar plugs (6mm) of fungal culture were inoculated in PDB for seven days at 28°C. After incubation, 5ml of culture filtrate was centrifuged at 10,000 rpm for 5 min and 1 ml of supernatant was taken in different test tubes in triplicates. The supernatant was mixed with 2 ml of Salkowski reagent [20] and allowed to react for 30 min in the dark. PDB without L-tryptophan was used for comparison. Development of pink-red colour showed positive results for IAA production. Quantitative estimation was done by UV-vis spectrophotometer at 530 nm and the quantity of IAA was calculated by the standard graph.

2.3. Standard Assays of Indole Acetic Acid

Different concentrations (25, 50, 100, 150, 200, 250 and 300 µg/ml) of standard IAA (HiMedia) were prepared in distilled water. Salkowski reagent was added to different dilutions of IAA (1:2) followed by the addition of two drops of orthophosphoric acid and kept in dark. After the development of pink colour in each tube, UV absorbance was observed at 530 nm to develop the standard curve [20].

2.4. Optimization of Indole Acetic Acid Production

Optimization of fungal IAA production was done at different ranges of pH, temperature, incubation time and rpm. A medium component such as tryptophan concentration, carbon sources and nitrogen sources were also optimized in the basal medium. The quantity of fungal biomass and IAA were analyzed after the incubation period.

2.5. Yield Enhancement of Indole Acetic Acid Using Optimized Parameters

All selected environmental parameters and medium components by one-time variable method for enhancement of fungal biomass and IAA production were maintained in basal medium. Maximize IAA production by *T. trachyspermus* was calculated by standard method.

2.6. Partial Purification and Characterization of Indole Acetic Acid

Extraction of crude IAA was done by the standard method [21]. Thin-layer chromatography plates (silica gel GF 254 thickness 0.25 mm) were loaded with crude IAA fraction (10-20µl) and standard IAA. The loaded TLC plate was kept in the mobile phase with the composition of benzene: n-butanol: acetic acid in 70:25:5 proportion for separation. Separated spots of IAA were visualized by spraying the plates using the Salkowaski reagent and Rf values of detected spots were calculated. Silica gel column chromatography was used for partial purification of IAA from crude extract and fractions were dissolved in a solvent system of ethyl acetate and hexane (20:80 v/v). Each fraction was tested in thin layer chromatography and then developed with Salkowski reagent. HPLC analysis of IAA was done on a C18 column (5µm; 25 x 0.46cm) by using an HPLC grade acetonitrile-water system

containing 0.1% trifluoroacetic acid was programmed over 30 min at a flow rate of 0.5mL/min with UV detector at 220 nm at 40°C. The mobile phase consisted of methanol and water (80: 20 v/v) run at flow rate was analyzed by comparison with the elution profiles of those authentic IAA injected separately [21].

3. Result and Discussion

3.1. Effect of pH on Indole Acetic Acid Production

IAA production by microorganisms varies from species to species and is greatly impacted by cell growth stage, availability of substrate and different environmental conditions [22]. *T. trachyspermus* was recognized for its immense IAA production capacity. Evaluation of IAA at different pH was showing maximum production of 64.89±2.4 µg/ml at 6.5 pH. Minimum production of IAA 2.92±0.69 µg/ml was found at 9.5 pH and 5.17±0.82 µg/ml was found at 2.5 pH (Figure 1). The dry weight of fungal biomass was higher at 7.5 pH 1.36±0.06 g/100ml and minimum at 0.06±0.05g/100ml (Figure 1). Mostly microorganism shows maximum IAA production at the neutral pH range. *Aspergillus niger was* reported for the production of IAA at pH 6 [23]. Many yeast species such as *candida sp.* showed their maximum production of IAA at neutral to slightly acidic pH [24]. Similarly, the *Trichoderma harzianum* InaCC F88 strain showed maximum production of IAA at pH 6.0 and maximum dry biomass at pH 8.0 [25].

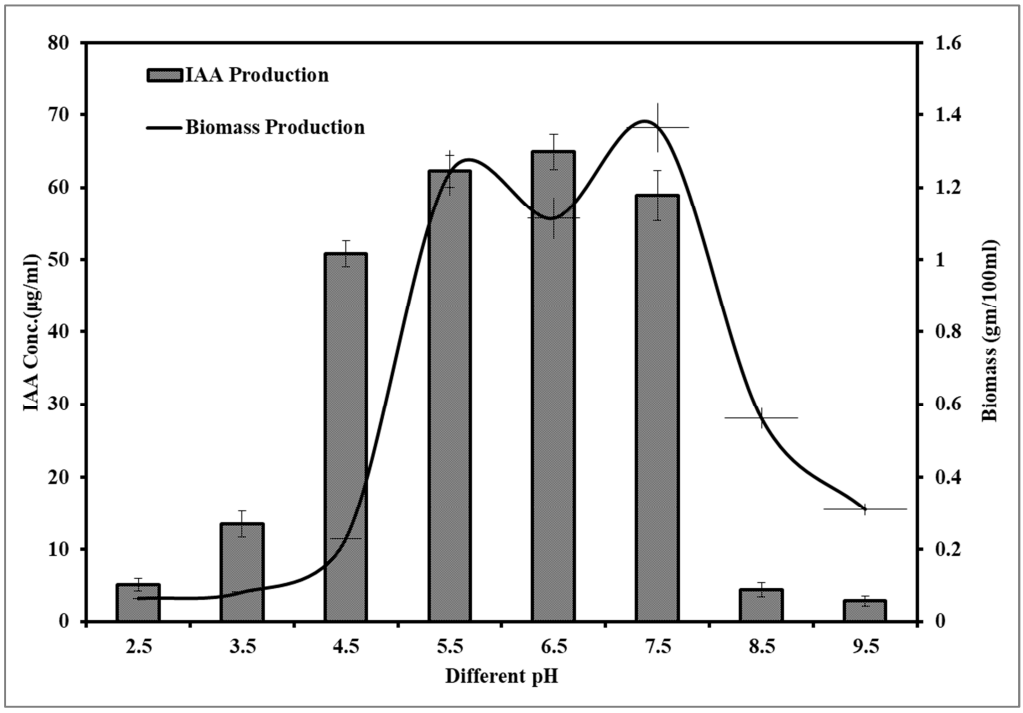

Figure 1. *Effect of different pH on IAA production and Biomass of Talaromyces trachyspermus.*

IAA production by microorganisms is greatly influenced by different pH because this environmental factor affects the enzymes involved in the biosynthesis of IAA. Amino acids of enzymes ionize by environmental pH and the activity of enzymes is affected due to conformation changes at active sites. In addition, the accumulation of hydrogen ions and the presence of cationic metal alter the shape of the enzyme and charge properties of the substrate involved in the metabolism of IAA biosynthesis. Due to this, enzymes and substrate are unable to bind that limiting the overall catalysis reaction. Hence, an enzyme can act only at its optimum pH [25].

3.2. Effect of Temperature on Indole Acetic Acid Production

The effect of different temperatures on fungal IAA production is shown in Figure 2. Out of all temperature ranges, maximum production of IAA 72.51±1.57µg/ml was found at 28°C. Minimum production of IAA 10.4±0.7 µg/ml by fungal culture was found at 19°C. The dry weight of fungal biomass was higher at 31°C (1.32±0.05 g/100ml) than at 28°C (1.22±0.07 g/100ml). Similar results were reported that the biomass of *Trichoderma harzianum* at the temperature of 27°C was lower (34.45 mg) than the higher temperature of 30°C (89.73 mg) [25]. Temperature affects the IAA production and the amount of fungal biomass. The sensitivity of *Talaromyces trachyspermus* towards temperature is shown clearly in this experiment. Enhancement of 3°C above 31°C, IAA production and dry weight of biomass of fungus gradually decreased. According to some recent studies optimum temperature range for all metabolites production of *Talaromyces sp.* is 25°C-30°C [18]. It is reported that 25°C and 30°C are the optimum temperatures for IAA production by yeast species [26]. It has been seen in investigations, that the optimum temperature range of microorganisms favours their IAA production. IAA

production has directly correlated with the growth ability of microorganisms at particular temperatures [25].

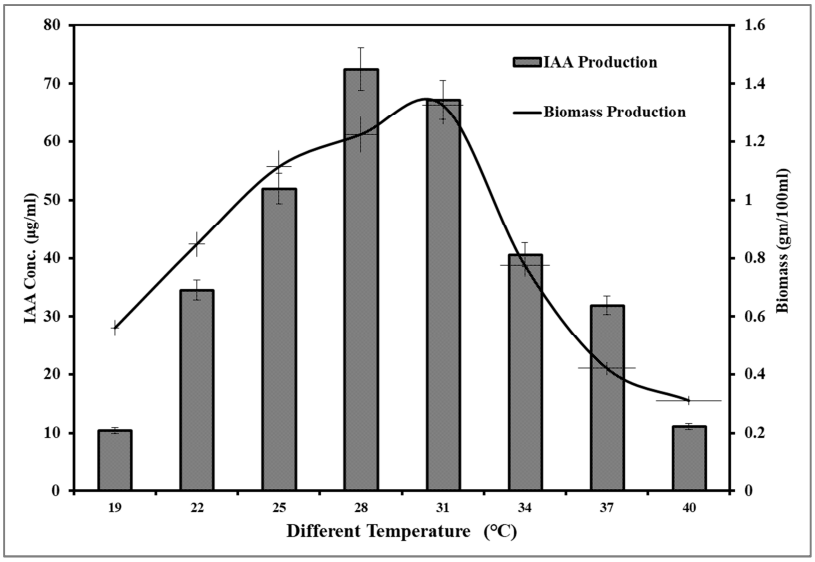

Figure 2. *Effect of Different Temperatures on IAA production and Biomass of Talaromyces trachyspermus.*

3.3. Effect of Incubation Period on Indole Acetic Acid Production

The effect of incubation time on IAA production was studied and maximum production of 68.22±0.9 µg/ml was found on 15 days of incubation time (Figure 3). IAA production was found to be in decreasing order by increasing the time duration. The dry weight of fungal biomass followed a similar trend and also peaked at 15 days (Figure 3). It has been seen in the axenic culture of *Tricholoma vaccinum*, a spruce ectomycorrhizal fungus, maximum IAA production is found for up to 28 days [27]. Immobilized cells of *Arthrobacter agilis* showed a gradual increase in IAA production from 6 to 24 h [28]. IAA production in fermentation broth increased up to a particular incubation time, then it gradually decreased might be due production of some enzymes i.e. IAA oxidase and peroxidase [14].

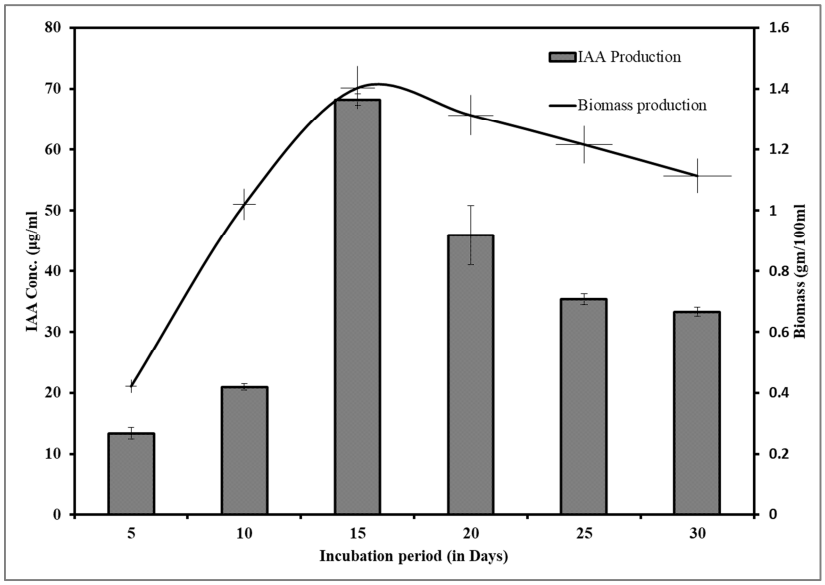

Figure 3. *Effect of different Incubation periods on IAA production and Biomass of Talaromyces trachyspermus.*

3.4. Effect of RPM on Indole Acetic Acid Production

Metabolite production by microorganisms depends on their oxygen requirement and nutrient availability. Optimum shaking condition prevents cell death and microbial clump formation in fungal liquid cultures by the uniform distribution of oxygen and nutrients [29]. Optimization of RPM is necessary for maximum growth and metabolite production by fungal cells. Different RPM (80, 100, 120, 140 160, 180 & 200) were adjusted for determination of the impact of the same

on IAA production by *T. trachyspermus.* Maximum production of IAA (69.70±1.69 µg/ml) by fungal culture was found at 140 RPM and it gradually decreases with an increase in shaking conditions (Figure 4). IAA production at 140 RPM is 1.89 times higher than 80 RPM, shaking condition was found to improve the effect on IAA production. The dry weight of fungal biomass was higher (1.31±0.05 g/100ml) at 120 RPM and it is also minimized with vigorous shaking (Figure 4). Different studies also reported that microbial IAA production is improved under shaking conditions than static [30, 31].

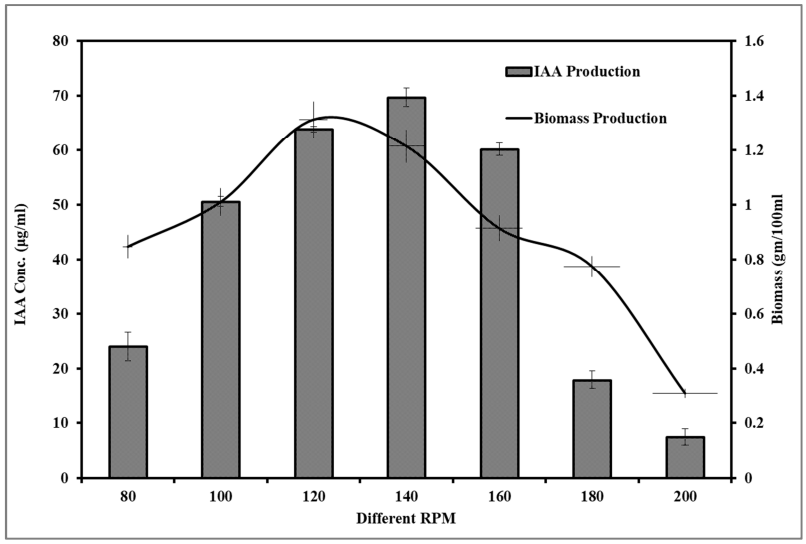

Figure 4. *Effect of different RPM on IAA production and Biomass of Talaromyces trachyspermus.*

3.5. Effect of Tryptophan Concentration on Indole Acetic Acid Production

Different concentrations of L-tryptophan (0.0% to 2.5%) were used for optimization of fungal IAA production. This experiment showed gradual enhancement of IAA production with an increase of L-tryptophan concentration up to 0.5%. After reaching a maximum production of 62.12±0.18 µg/ml at 0.5% of L-tryptophan IAA production was decreased. Very slight changes occurred in the dry weight of fungal biomass with different L-tryptophan concentrations. However, maximum fungal biomass of 1.30±0.06g/100ml was found at 1.5% (Figure 5). *Trichoderma harzianum* showed approximately the same biomass profile at different concentrations of L-tryptophan [25]. Results showed that the production of IAA by *Talaromyces trachyspermus* is L-tryptophan dependent. *Trichoderma harzianum* WKY1 fungus produced 5 times more IAA in the presence of 0.1% L-tryptophan than in the medium without the precursor [31]. In the same L-tryptophan concentration (0.1%) our culture showed 8.5 times enhancement of IAA.

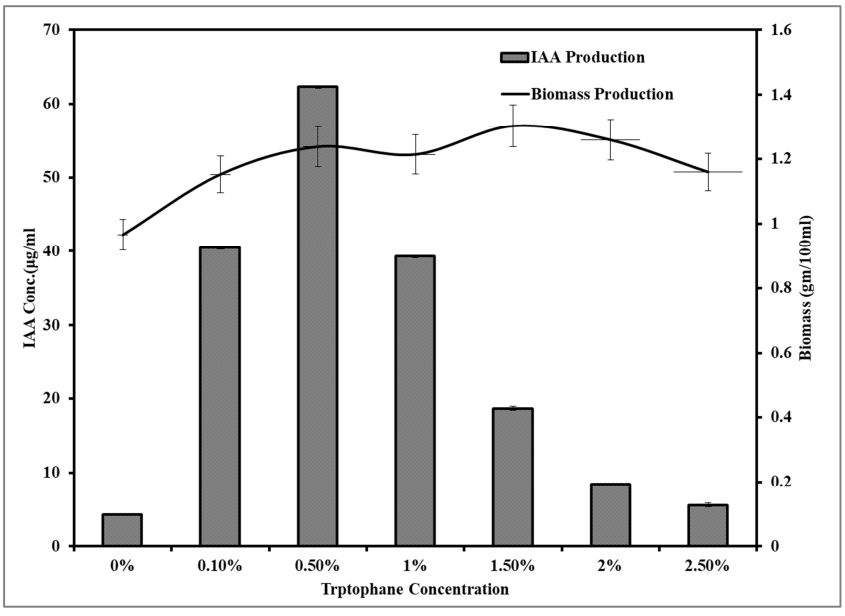

Figure 5. *Effect of different L-Tryptophan concentrations on IAA production and Biomass of Talaromyces trachyspermus.*

3.6. Effect of Carbon Sources on Indole Acetic Acid Production

The Carbon source is one of the significant parameters for the production of the metabolites by microorganisms. It affects the overall efficiency of the biosynthesis of IAA and biomass production by microbes [32]. Eight different carbon sources were used for analyzing their effect on IAA production by *T. trachyspermus.* Among the eight carbon sources, glucose was found to show the maximum IAA production 63.73±0.17 µg/ml and dry weight of biomass 1.41±0.05 g/100ml (Figure 6). Different concentrations of glucose (2%, 2.5%, 3%, 3.5%, 4%, 4.5% & 5%) were also analyzed. Maximum IAA 64.73±0.17 µg/ml and biomass content 1.33±0.05 g/100ml were found in the medium amended with 4% glucose (Figure 7). Glucose is the basic carbon source and is easily available for fast cell growth and metabolites production. It has been also reported that *Rhizobium spp*. produce maximum IAA and biomass in the presence of glucose [33]. Different studies suggested that various carbon sources and their concentrations in the basal medium affect the production of IAA and growth of microbes differently [30, 34, 35].

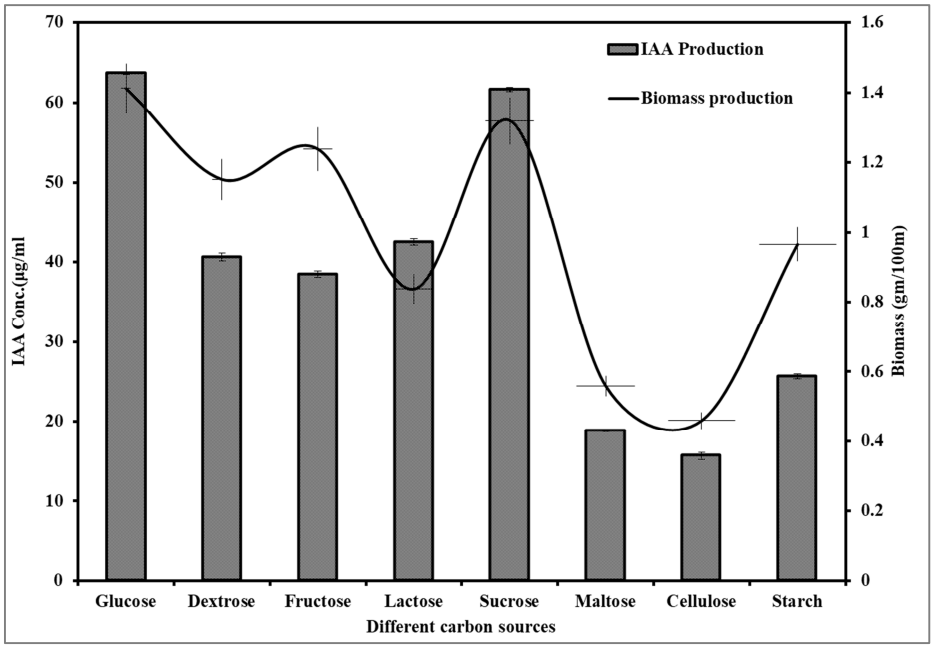

Figure 6. *Effect of different Carbon sources on IAA production and Biomass of Talaromyces trachyspermus.*

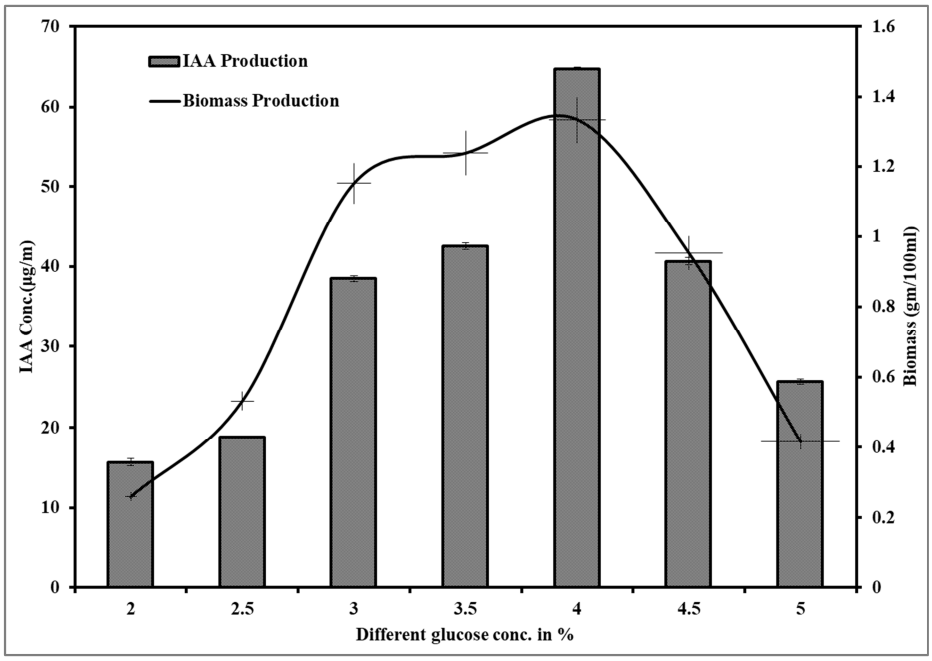

Figure 7. *Effect of different concentrations of glucose on IAA production and Biomass of Talaromyces trachyspermus.*

3.7. Effect of Nitrogen Sources on Indole Acetic Acid Production

Nitrogen is an essential component of the cell because it is the fundamental element of proteins, nucleic acids and cell walls. The growth and cellular metabolism of microorganisms are directly affected by the source of nitrogen present in the culture medium [36]. Our fungal culture was tested against eight different nitrogen sources and maximum IAA production of 63.73±0.17 µg/ml was found in the presence of sodium nitrate. This nitrogen source was also found for the highest fungal biomass (1.44±0.04 g/100ml) production (Figure 8). In this study, fungal culture was showing its best results in inorganic nitrogen sources than organic sources. Different concentrations of sodium nitrate were also tested and 0.1% was found for the best results (IAA 63.73±0.17 µg/ml and biomass 1.22±0.05 g/100ml) (Figure 9). IAA production and biomass of fungus were gradually decreased with increasing the concentration of sodium nitrate in the medium. Studies reported that different nitrogen source and their concentrations affect cell growth and IAA production [30, 35].

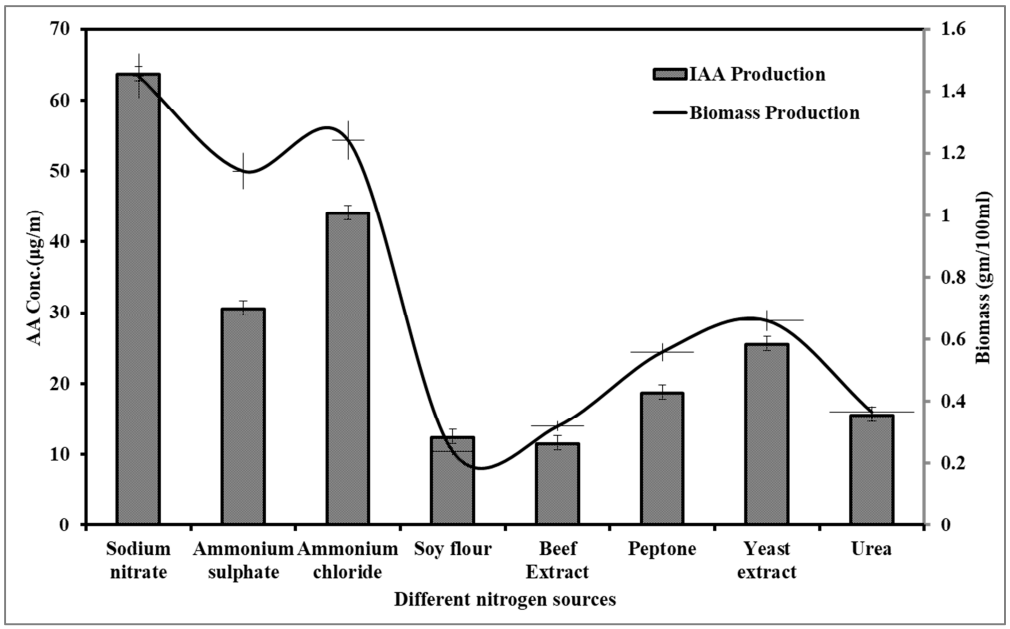

Figure 8. *Effect of different Nitrogen sources on IAA production and Biomass of Talaromyces trachyspermus.*

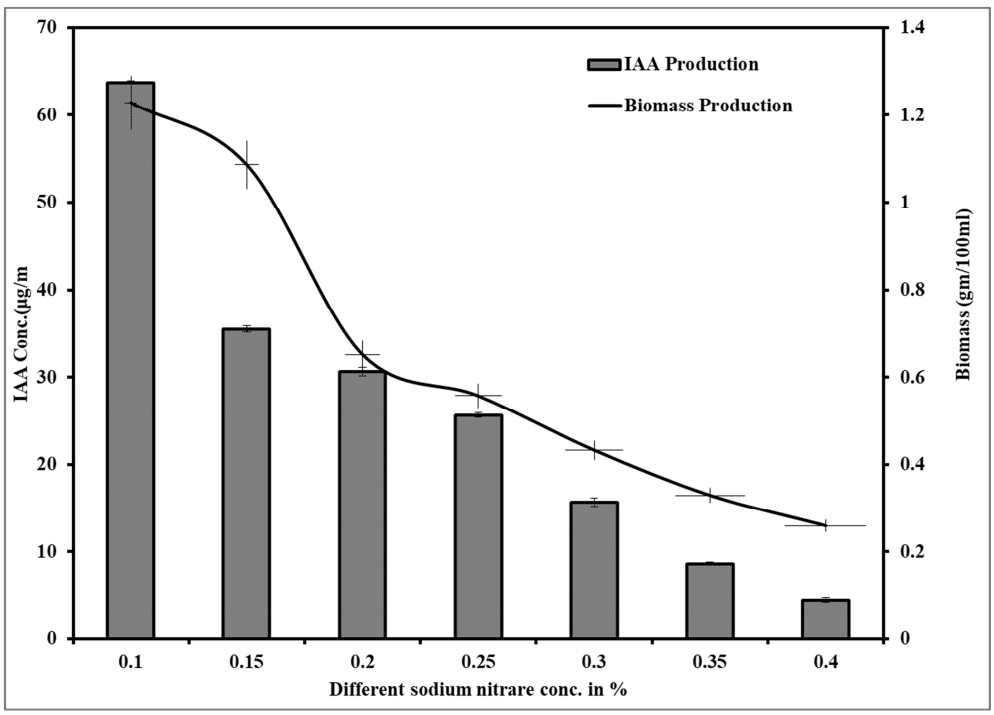

Figure 9. *Effect of Different Concentration of sodium nitrate on IAA production and Biomass of Talaromyces trachyspermus.*

3.8. Indole Acetic Acid Production Under Optimized Conditions

Optimization of IAA production by *T. trachyspermus* was done at different pH, temperature, incubation time, RPM, tryptophan concentration, carbon sources and nitrogen sources. After optimization, the yield of IAA by fungal culture was increased up to 0.78 fold as compared to initial production. Fungal culture was producing 61.34±0.23 µg/ml of IAA under non-optimized parameters while after optimization IAA production was 109.25±0.19 µg/ml.

3.9. Characterization of Indole Acetic Acid

Characterization of IAA produced by *T. trachyspermus* was done by TLC and HPLC methods. The crude fungal extract showed a clear pink colour spot on the TLC plate and an Rf value corresponding to standard IAA (0.62) (Figure 10). Similar studies were also reported for endophytic *Bacillus spp.* from *Vigna radiate* [37]. Results of HPLC analysis showed that the peak of the crude fungal extract is at a similar retention time (4.96) to standard IAA (Figure 10).

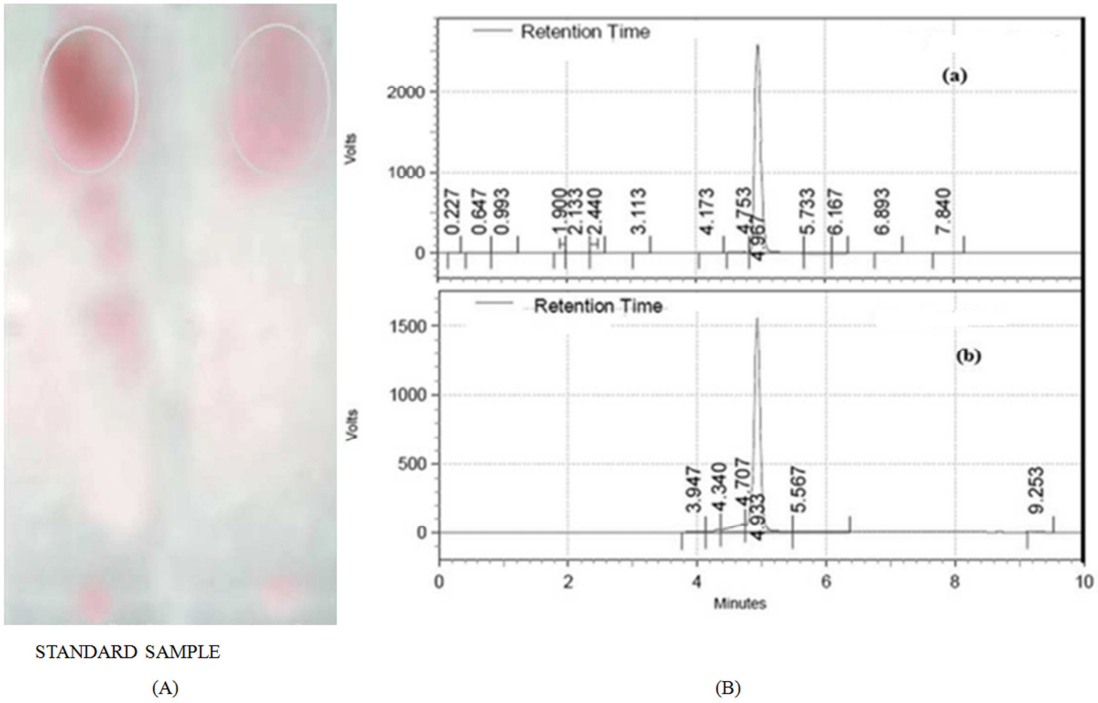

Figure 10. *(A) TLC Profile (B) HPLC profile of crude extract of Talaromyces trachyspermus.*

4. Conclusion

The present study demonstrates that *Talaromyces trachyspermus* is a potent plant growth-promoting fungus and has the immense efficiency of Indole acetic acid secretion. The production of fungal Indole acetic acid was enhanced up to 0.78 fold after optimization of various environmental parameters. There is an emerging demand for potent bio inoculants and their bioactive metabolites for economic and environmentally safe agriculture. Bioactive metabolites of plant growth-promoting microorganisms such as IAA are used as the quick and safe mode of growth promoters in modern agro-practices. Optimization of biosynthesis pathways of microbes by modulating various environmental conditions can enhance the yield of metabolites. Further studies are needed to explore the genetic modulation in the biosynthesis metabolic pathways of IAA in potent microbes for more production of this secondary metabolite. After that, we can ensure the potency of plant growth-promoting microbes as bio inoculants and as agro-industrially important strains.

This study reveals the impending potential of *Talaromyces trachyspermus* for the development of biofertilizers as well as a promising producer of IAA in the industry. Production of IAA in industry or use of plant growth-promoting fungus in agriculture field not only enhances the crop production but also supports the eco-friendly agriculture practices.

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Conflict of Interest

The authors declare that they have no competing interests.

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