J. Mycopathol. Res. **61(2)** : 201-206, 2023; ISSN : 0971-3719 (Print), 2583-6315 (Online) doi. 10.57023/JMycR.61.2.2023.201

Trichoderma species: screening and *in vitro* evaluation of its plant growth promoting potential

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Received: 11.02.2023 Accepted:20.04.2023 Published: 26.06.2023

Two *Trichoderma* isolates were isolated from soil samples collected from various vegetable and fruit crop fields of Satana region, District Nashik. Morphological and microscopic characters of isolated fungi were studied. Plant growth promoting activities like indole acetic acid production was evaluated. *Trichoderma* is best known for its biocontrol activities and the production of antifungal compounds. Recent research supported the hypothesis that isolated *Trichoderma* species produce the plant growth promoter Indole-3-acetic acid (IAA). The isolate UGK-1 produced the maximum amount of indole-3-acetic acid at 118 µg/ml. During production, the amount of IAA is elevated for 96 h, then it decreased after120 and 144 h of incubation. Ability of production by native strains of *Trichoderma* confirmed by using medium, potato dextrose broth and In vitro plant trials including *Vigna radiata* and *Vigna unguiculata*, seed germination increased by 98 and 96 percent, respectively. Shoot length and root proliferation were also significantly increased.

Key words: Indole-3-acetic acid (IAA), *Trichoderma, Vigna radiata, Vigna unguiculata*

INTRODUCTION

Fungal Genus *Trichoderma* belongs to the class Deuteromycetes, which is a filamentous, ascomycetous, asexual spore producing fungus. *Trichoderma*, a fungus principally present in soil, has proven to play a significant role in agriculture due to its wide application. Over the last few decades, several studies on various aspects of its solidarity roles have been conducted.

It is a prime attraction for most agricultural industries and farmers because of its diverse applications in enhancing agricultural productivity. It is a potential competitor found in the rhizosphere soil of most plants and an effective biocontrol agent against many plants pathogenic fungi (Rahman, *et al.* 2021). Members of the *Trichoderma* fungi are regarded as potential biocontrol agents because they may produce varieties of products like peptaibols, gliotoxin, gliovirin, polyketides, pyrones, and terpenes (Vinale,2008; Leylaie and Zafari 2018). *Trichoderma* species may be able to produce the auxin phytohormone, indole-3-acetic acid (IAA) (Nieto-Jacob, 2017), which is the endogenous auxin that controls many processes in plants, including apical dominance, tropism, shoot elongation, root initiation cell division, cell expansion, cell differentiation, and fruit development (Matsuda *et al.* 2005). Being vital to plants, IAA attracted a great deal of attention. IAA might involve proliferating the network of root systems of plants, which enable the plant's nutrient absorption region in the rhizosphere soil and also enhance the microbial population near the root system (Nieto-Jacob*,* 2017; Spaepen *et al*. 2007; Berg 2009; Contreras-Cornejo *et al*. 2009).

The goal of this study was to screen for desirable *Trichoderma* species in soil samples collected from local regions in Satana, Nashik (India). Two species of *Trichoderma* were isolated by studying morphological and microscopic characters. After purification of the isolates, they were tested for the production of growth promoting potential by employing an *in vitro* plant study.

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MATERIALS AND METHODS

Collection of rhizosphere soil samples

Soil samples were collected from the rhizospheres of five different vegetable and fruit crop plants, such as tomato, spinach, onion, pomegranate, and grape. Some of the plants were uprooted, and the soil was collected in sterile plastic containers. Collected soil samples were brought to the laboratory and air dried to remove some moisture before being used for further study.

Screening of Trichoderma species

For screening of desirable *Trichoderma* species, the following media were used:

Potato dextrose broth: Prepared potato extract as: 20 grams of potato mixed in 100 ml of distilled water and boiled for 30 min and filtered. Then dextrose was added at a concentration of 2 g/L. To inhibit bacterial growth, 0.02 g of chloramphenicol was added. The medium was acidified by lowering the pH to 3.5. This dehydrated medium was stored at a low temperature in a tightly closed container and used for further medium preparation.

Rose Bengal broth and potato dextrose broth as enrichment media for screening of *Trichoderma* Sterile Rose Bengal medium containing g/L peptone (5.0 g), Rose Bengal (0.05 g), glucose (10.0 g), chlortetracycline (0.1 g), dipotassium phosphate (1.0 g), and MgSO4.7H20 (0.5 g) was used for the isolation of *Trichoderma* species. The antibiotic chlortetracycline was added to suppress the growth of bacteria. Initially, the liquid enrichment culture technique was used for fungi enrichment.

In 100-ml flasks containing 50 ml of Rose Bengal Broth and Potato Dextrose Broth, 1g of soil samples was inoculated. Each flask was incubated at room temperature until visible growth appeared in the medium. After the appearance of visible growth in flasks, small aliquots were transferred onto potato dextrose agar plates by streaking the plates. Plates were incubated at room temperature until desirable fungus growth appeared. Then, a pure culture of isolates was obtained by transfer and retransfer onto fresh PDA plates; the pure culture was maintained on PDA slants and maintained at a low temperature.

Morphological characterization

For morphological analysis, strains were grown on PDA at 27 °C for 5-7 days. Microscopic observations were done using a binocular microscope. Conidiophore structures and morphology were examined. Conidial morphology and size were recorded after 7 days of incubation. *Trichoderma* species were identified according to Bissett (1991) and Siddiquee (2017). The colony morphology of each *Trichoderma* isolate was recorded. For microscopic observations, specimens were prepared. Using a binocular microscope, the spore shape, size, and mycelial arrangement of each isolate were studied. For the study, a specimen of the isolate was prepared, and lactophenol cotton blue staining was performed as a tease mount. On a grease-free, clean glass slide, a drop of lactophenol blue was placed, and the fungal specimen was added over this drop of lactophenol blue by using a sterile inoculating wire loop. With the help of a sterile dissecting needle, the specimen was gently teased so that it would spread out in the lactophenol. The mixer of lactophenol and fungal specimens was then covered with a clean coverslip, which was slowly lowered to avoid trapping air bubbles under the coverslip. After this procedure, the specimen was observed under low- and high-power magnification lenses of a microscope. Observations were recorded.

Evaluation of production of Indole-3-acetic acid by isolates

Methods of Gordon and Weber (1951), Gang *et al.*(2019) and Ratnam (2020) were followed with modifications for evaluation of IAA production by isolates. The inoculum of an isolated strain was inoculated in potato dextrose broth and incubated at room temperature on an orbital shaker at 120 rpm for 2 days. After incubation, the culture broth was centrifuged at 5000 rpm for 15 min. 1 ml of supernatant was mixed with 1 mL of Salkowski reagent and incubated for 30 min at room temperature. The appearance of the pink colour demonstrated IAA production. Absorbance was measured at 530 nm. In order to perform the quantitative assay for IAA production, an isolated strain of *Trichoderma* was inoculated in 200 ml of potato dextrose broth supplemented with tryptophan at a concentration of 0.5 g/L. The culture broth was incubated at room temperature

on an orbital shaker at 120 rpm for 6 days. After an interval of 24 hours of incubation, the broth was separated from the biomass of *Trichoderma*, initially by filtration and then by centrifugation at 5000 rpm for 15 min. A standard curve for estimation was created by taking pure indole-3-acetic acid in different concentrations and using Salkowski's reagent. Optical density was measured at 530 nm.

In vitro trial for growth promoting potential of isolates

The isolate's growth-promoting potential was assessed using fresh seeds. The dry seeds of local varieties of *Vigna radiata* and *Vigna unguiculata* were taken for the experiment. The seeds of both plants are washed in sterile water three times. The soil was collected from a botanical garden. The soil was first air-dried in the shade for 3 days and then filled into plates. The dried seeds of both varieties were sown in the soil. The water was sprinkled to maintain the moist conditions. Plates were kept in the laboratory near the light. All test plates were applied with crude supernatant taken from the production medium to evaluate the growth-promoting potential of isolated *Trichoderma* species. All plants were grown for 8–10 days. Observation and evaluation of different parameters such as germination percentage, shoot length, and root proliferation were recorded.

RESULTS AND DISCUSSION

Enrichment of sample

Trichoderma species were isolated from soil by using liquid and solid enrichment methods. During isolation, rapid growth was observed, indicating that fungi have a high growth ability. After incubation, PDB showed visible growth in each flask (Fig.1). Further purification of enriched samples was conducted for each isolate.

Growth of isolates on PDA plate

The visual growth from each flask was streaked and re-streaked on PDA plates for further purification, and finally a spot inoculated at the centre of the plates. After incubation, the isolated organism showed growth similar to that of *Trichoderm*a species. Initial growth of the isolate was observed at the centre of the plate. Growth began as cottony white, then turned brownish-yellow, and finally dark green. Isolates grew first in the centre of the plate, then in the surrounding medium. Further, it was elaborated in a concentric fashion, as shown in (Fig.2 A&B). The growth of all isolates was studied on PDA plates with a similar approach.

Study of morphological characters of isolates

Lactophenol cotton blue staining was performed, and the mycelial pattern of growth was observed. Some characteristic patterns of fungal hyphae were observed. *Trichoderma* strains are frequently identified by morphology, which includes rapid growth and bright green or white conidial pigments (Siddiquee, 2017). Extensive review of reports on *Trichoderma* species, the isolates discovered contained parts such as conidia of various morphologies, white at first, then green later. Conidia and chlamydospores are present in most species in varying size and numbers. Hyphae of fungi generally have a potential network containing phialides, conidia that are green (rarely brownish), and non-septate hyphae. The principal two isolates that resembled *Trichoderma* species that were screened out showed the presence of the typical conidial pattern and short phialides as well. The patterns of conidiophore branching and aggregation of conidiophores into full-fledged mycelium, which are characteristic features of strains of *Trichoderma* species (Siddiquee 2017). Microscopic observation of isolated strains of fungi revealed a predominant network of mycelium, and conidia appeared on the terminals of conidiophores, which bear a resemblance to species of *Trichoderma* as shown in (Fig. 3 A&C). The phenetic identification of isolates is awaited. Conidial structure of both isolated *Trichoderma* species, which had green pigmentation in round to oval-sized conidia have been presented (Fig.3 B&D).

Production of growth hormone

The production of plant growth hormone by isolated *Trichoderma* species was evaluated. Following incubation of *Trichoderma* species in production media (potato dextrose broth with tryptophan), a small volume of the sample was removed from the culture and processed for indole-3-acetic acid estimation. Production of indole-3-acetic acid using an isolate of *Trichoderma* species UGK-1 has been

Table 1. Production of IAA by *Trichoderma* isolate UGK 1

Time of Production of IAA (Hours)	Amount of IAA produce (µg/ml)
24	26
48	43
72	58
96	118
120	82
144	28

Table 2. Assessment of parameters during *in vitro* trials

Plant Variety	Germination percentage	Average Shoot length (cm)		Root proliferation (Average number of rootlets)	
		Control	Test	Control	Test
Vigna radiata	98	13	21	6	16
Vigna unguiculata	96	12	19	4	17

Fig. 1 : Enrichment of soil samples

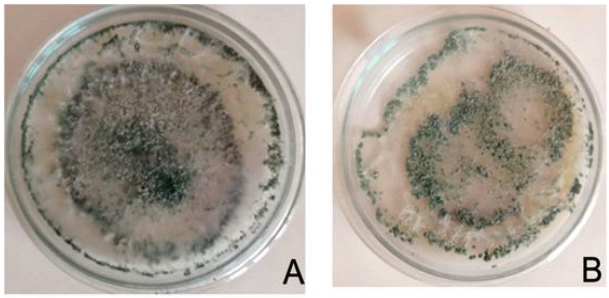

Fig. 2 : Growth of *Trichoderma* isolates - (A) UGK 1 and (B) UGK 2 in Petri plates

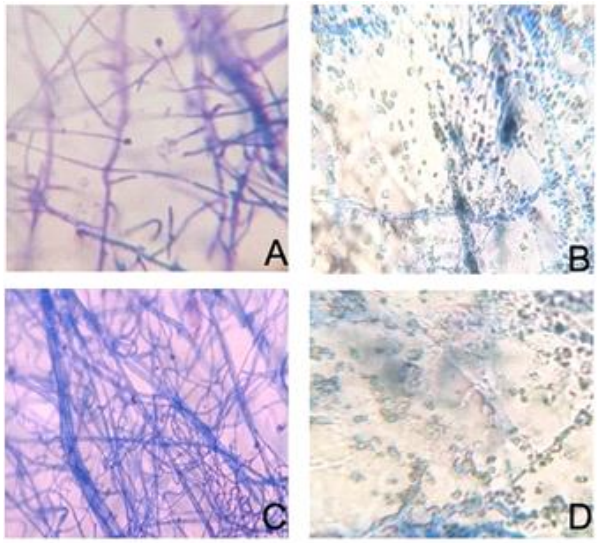

Fig. 3 : Microscopic observation of (A & C) Mycelia and (B & D) conidia of *Trichoderma* isolates UKG 1 (A & B) and UKG 2 (C & D).

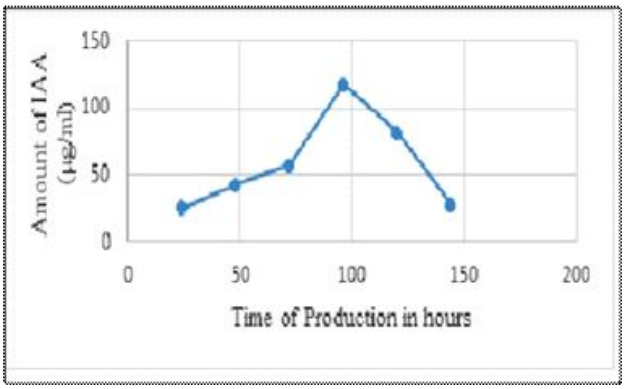

Fig. 4 : Production of IAA by *Trichoderma* isolate UGK-1

Fig. 5: Influence of IAA on plant growth during *in vitro* trial

evaluated. The maximum amount of indole-3-acetic acid produced by isolate UGK-1 was 118 µg/ml. Production was conducted for six days. During production, it was noted that the amount of IAA was elevated until 96 hours, then it decreased at 120 and 144 hours of incubation (Table 1; Fig. 4).

In vitro evaluation of the growth-promoting potential of isolates

All plants were grown for 8–10 days. Periodically, observations about different parameters such as germination percentage, shoot length, and root proliferation were recorded. Results have been presented in Table 2 and Fig. 5.

CONCLUSION

Trichoderma species, a class of plant-beneficial fungi, may provide opportunistic symbionts to induce plant tolerance against various factors. In this study, a total of 2 isolates of *Trichoderma* species were isolated from the rhizosphere soil of vegetable and fruit plants. The maximum amount of indole-3-acetic acid, i.e., 118 µg/ml was recorded at 96 hours of production time by isolate UGK-1. The optimal time required for the production of the maximum amount of IAA is 96 hours. Different parameters, such as germination percentage, shoot length, and root proliferation, were evaluated in plant trails. Seed germination for both plants, *Vigna radiata* and *Vigna unguiculata*, increased by 98 and 96 percent, respectively. Shoot length and root proliferation were also significantly increased.

ACKNOWLEDGEMENTS

The authors are thankful to the Principal and Head of the Department of Microbiology, KAANM Sonawane ASC College, Satana, for providing laboratory facilities to carry out the research work and their inspiring help.

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