Plant & Fungal Research (2025) 8 (1): 11-22
https://doi.org/10.30546/2664-5297.2025.8.1.06

June 2025

Plant growth-promotion and antipathogenic fungal activity of four rhizosphere isolates of *Trichoderma* from southwest India

Hosamane Kavya
Department of Post-Graduate Studies in Biotechnology, Alva's Autonomous College, Moodbidri, Karnataka, India
Ali Jawad Akki
Department of Biotechnology, School of Applied Science and Technology, Bijapur Lingayat District Educational Association (Deemed to be University), Vijayapura, Karnataka, India
Raghavendra Rao Badkillaya
Department of Post-Graduate Studies in Biotechnology, Alva's Autonomous College, Moodbidri, Karnataka, India
Santhosh Wilson Goveas
Department of Biotechnology, St. Aloysius (Deemed to be University), Mangalore, Karnataka, India
Kandikere Ramaiah Sridhar[1]
Department of Biosciences, Mangalore University, Mangalagangotri, Mangalore, Karnataka, India

Abstract: *Trichoderma* has the potential to promote plant growth and is antagonistic to phytopathogenic fungi. Four *Trichoderma* strains were isolated from the rhizospheres of banana (*Musa acuminata*), nutmeg (*Myristica fragrans*), pepper (*Piper nigrum*) and tamarind (*Tamarindus indica*) (AMCC 1-4). Their culture extracts were assessed for seed germination, radical growth in mung bean (*Vigna radiata*), and root and corm development in the endangered species elephant foot yam (*Amorphophallus paeoniifolius*). The culture filtrate of all *Trichoderma* isolates significantly increased seed germination as well as enhanced the radical growth of mung bean (*Vigna radiata*), with the highest germination and radical growth by the AMCC 3 (90%). The AMCC 3 also enhanced root growth in yam (10 vs. 20 cm), while corm density increased by AMCC 2 (5 vs. 10). The analysis by high-performance liquid chromatography (HPLC) of culture filtrate of the AMCC 3 showed the presence of indole-3-acetic acid (IAA) (11.5 µg/ml). All isolates of *Trichoderma* possess phosphate solubilization, with the highest solubilization index by AMCC 1 (1.4). Culture filtrates of all *Trichoderma* isolates showed significant in vitro growth inhibition of the phytopathogen *Agroathelia rolfsii*, with the highest inhibition by the AMCC 3 (60%). On culturing *Trichoderma* isolates on carboxymethyl cellulose (CMC) agar for up to four days, all isolates showed a clear zone around the colonies confirming their cellulase-producing ability, with the highest production from the AMCC 3 (31.3 mm). The internal transcribed spacer (ITS) gene from AMCC 3 was sequenced and compared with the NCBI database using the BlastN program. The DNA sequence of AMCC 3 corresponds with that of *Trichoderma erinaceum*. This study reveals that four *Trichoderma* isolates from the rhizosphere of four horticulture plant species possess the plant growth promotion ability as well as growth inhibition of the phytopathogen *Agroathelia rolfsii* with high efficiency by *T. erinaceum* (AMCC 3). It qualifies to be adapted as a plant growth stimulant and biopesticide against fungal phytopathogens in lateritic loamy soils of southwest India.

Keywords: *cellulolytic activity, growth inhibition, indole acetic acid, phosphate solubilization, radical growth, Trichoderma erinaceum*

INTRODUCTION

According to the United Nations Food and Agricultural Organization (FAO), about 20-40% of crop devastation in developing countries occurs mainly due to diseases and pests [United Kingdom Food Security Report, 2021]. Among the total crop failure due to pests, up to 12% is mainly due to the attack of phytopathogens leading to severe economic losses [Sharma et al., 2012; Saldaña-Mendoza et al., 2023]. Management of diseases in economically important crops is of vital importance for sustaining the quantity as well as the quality of crop production. Among the different strategies of disease control, the deployment of chemicals is popular in most of the regions and many disease control chemicals are applied at high doses in an unscientific manner [He et al., 2021; Pandit et al., 2022]. Most of the toxic chemicals that are used by farmers for controlling diseases are poisonous and profoundly pollute the environment [Damalas, Koutroubas, 2016; Naidu et al., 2021]. Nearly 20,000 people in developing countries are speculated to undergo death each year due to consumption of foods contaminated with pesticides [Abodu, 2018]. Indiscriminate use of pesticides is also responsible for increased cost of cultivation and development of resistance in several pests and pathogens.

[1]E-mail: kandikeremanasa@gmail.com
Received: 23.04.2025; Received in revised form: 30.04.2025; Accepted: 31.05.2025

Rahaman et al. [2011] reported that *Trichoderma* species are frequently present in all types of soil, manure and decaying plant tissues. Their dominance in soil may be attributed to their diverse metabolic capability and aggressive competitive nature [Elad, 2000]. Okoth et al. [2007] isolated 306 *Trichoderma* strains from 60 soil samples from different regions and observed *Trichoderma harzianum* to be the most frequently isolated species, but in coffee farms, *Trichoderma viride* was the most frequently identified species, while *Trichoderma citrinoviride* was the most common in Napier farms. Considering threats of chemical pesticides in plant pest and disease management, nowadays researchers are paying more attention to identifying alternative approaches that reinstate the use of hazardous chemical pesticides for controlling diseases and pests. Among the few powerful alternatives, biological control of diseases and pests is the most preferred, as it is safe, ecofriendly and cost-effective [Nakkeeran et al., 2004; Moser et al., 2008]. Till date, several biological control agents have been registered and are commercially available [Vinale et al., 2008]. Among them, various *Trichoderma* species are the most promising and extensively used biosystems in the growth promotion and biocontrol agent of crop plants against phytopathogens [Natsiopoulos et al., 2022; Tyśkiewicz et al., 2022; Saldaña-Mendoza et al., 2023]. They are well known for their mycoparasitism against a broad array of plant pathogens, especially those that live in soils, attacking roots and aerial parts of economically important crops [Chaube et al., 2002; Elad et al., 2000; Saldaña-Mendoza et al., 2023]. Different species of the genus *Trichoderma* grow rapidly in soil and rotting organic matter [Druzhinina et al., 2011]. Some species of *Trichoderma*, in addition to biological control, promote plant growth by their hormones [Kumar, 2013].

Trichoderma is known for its rapid growth, effective biofungicide and plant growth-enhancing potential [Natsiopoulos et al., 2022; Asghar et al., 2024]. This is very popular in the farming sector due to strong adaptability, eco-friendliness, low production cost and broad-spectrum activity against many phytopathogens [Sood et al., 2020]. They have been used as a biocontrol agent since their first application in the 1930s to control plant pathogens [Ha, 2010]. The *Trichoderma* controls plant pathogens by mechanisms such as the production of antibiotics, parasitism, and stimulation of defense in host plant species [Saldaña-Mendoza et al., 2023]. It has been observed that native isolates of *Trichoderma* perform better with respect to control of plant pathogens compared to non-native strains as they adapt very easily [Harman, 2011]. They also form associations with the host plant species quickly and exert effects like resistance to pathogens and promotion of plant growth [Jambhulkar et al., 2024]. In addition to biocontrol activity, *Trichoderma* is also known for its plant growth promotion potential [Stewart, Hill, 2014; Natsiopoulos et al., 2022]. The effectiveness of *Trichoderma* as a bioinoculant can be harnessed better if sufficient numbers of its isolates from different geographic locations are available [Naher et al., 2019]. The present study characterizes four *Trichoderma* isolates obtained from the rhizospheres of horticulturally important plant species for plant growth promotion and control of phytopathogens in southwest India for their future applications.

MATERIALS AND METHODS

Soil samples and isolation of Trichoderma. Rhizosphere soil samples from healthy plant species of banana (*Musa acuminata* Colla), nutmeg (*Myristica fragrans* Houtt.), pepper (*Piper nigrum* L.) and tamarind (*Tamarindus indica* L.) were collected in sterile polythene sachets, brought to the laboratory and processed to isolate *Trichoderma* strains following the serial dilution technique. One ml of aliquots of the 10^{-2} dilution was plated on *Trichoderma* selective medium [(KH_2PO_4, 0.9 g; NH_4NO_3, 1.0 g; $MgSO_4$, 0.20 g; KCl, 0.15 g; glucose, 3.0 g; Rose Bengal, 0.15 g; chloramphenicol, 0.25 g; streptomycin, 0.05 g; agar, 15 g; pentachloronitrobenzene (PCNB), 0.3 g; distilled water, 1 l)]. After the emergence of colonies on the medium, they were purified by the hyphal plugs from colony margins. Isolates of *Trichoderma* were confirmed based on morphological characteristics (Tab. 1). Fast-growing isolates from each plant species were selected for further study and they were deposited in Alva's Microbiology Culture Collections (AMCC).

Plant growth stimulation: Seed germination and radical growth. To test the impact of *Trichoderma* culture extracts on seed germination of green gram [(*Vigna radiata* (L.) R. Wilczek)], 25 healthy seeds were sown on Whatman № 1 filter paper spread on standard Petri plates. Ten ml of culture filtrate was added to each Petri plate, which was allowed to germinate for up to two days in the dark at laboratory temperature (28±2°C). After the second day, germinated seeds were scored to calculate the percentage of germination. Three replicates each of 100 seeds were maintained to test the culture filtrates. Seeds incubated in distilled water instead of culture

Table 1. Colony characteristics of *Trichoderma* isolates from four horticulture crops.

Trichoderma isolate №	Rhizosphere soil	Colony characteristics
AMCC 1	Banana	Initially, the colony colour was light green in the centre gradually becoming dark green on PDA medium.
AMCC 2	Nutmeg	Whitish to pale green mycelial mat uniformly grown in 3-4 days on PDA, which is gradually becoming whitish.
AMCC 3	Pepper	Initially, mycelia were whitish to greenish, gradually turning into deep green from the centre of the colony.
AMCC 4	Tamarind	Initially, the colony was whitish to light green, with watery in centre, gradually turning into deep grass green.

filtrate served as a control.

To assess seed growth stimulated by Trichoderma culture filtrates, a seedling bioassay of green gram was carried out [Garuba et al., 2015]. Seeds were surface sterilized by immersing them in ethanol (96%; 1 min) followed by sodium hypochlorite (6% available chlorine; 3 min) followed by ethanol (96%; 0.5 min) and three washes in sterile distilled water. Twenty-five seeds per Petri plate (10cm diam.) were treated with culture filtrates as described for seed germination in the dark at laboratory temperature (28±2°C). Surface-sterilized seeds soaked in filter paper-lined Petri dishes with distilled water served as a control. Radicle length of germinated seeds was measured using a slide calliper.

Growth promotion. The IUCN red listed threatened species, elephant foot yam tubers were washed thoroughly with running tap water, followed by household bleach (20%, 30 min), followed by three rinses in sterile distilled water. The basal region of surface-sterilized tubers was then dipped in the culture filtrates of *Trichoderma* isolates for up to one hour and the basal region was incubated touching the soil. Simultaneously, surface-sterilized tubers soaked in distilled water served as a control.

Detection of IAA. Trichoderma isolate AMCC 3 was grown on potato dextrose broth for up to 10 days at laboratory temperature (28±2°C), followed by filtering through filter paper (Whatman № 1). The filtrate was extracted with ethyl acetate and subjected to high-performance liquid chromatography (HPLC). Extracts were passed through the Sunfire C-18 column (5 µm, 4.6 × 250 mm) with a flow rate of one ml per min under isocratic conditions with a separation solvent (40% methanol and 60% of a 1% acetic acid) [Amina et al., 2017]. A 20 µl aliquot of extract was used for injection to the detector (228 nm and 280 nm). Data acquisition was performed by Empower 2 software (Waters Corporation, Massachusetts, USA). An AR-grade indole-3-acetic acid (IAA) was used as a standard (HiMedia Pvt. Ltd., Mumbai, India).

Phosphate Solubilization. Plugs of five-day-old *Trichoderma* isolates on PDA (5 mm diam.) were inoculated at the center of the Petri plate containing the Sperber's agar medium ($CaCl_2$, 10%; K_2HPO_4, 10%) [Sperber, 1957]. Phosphate solubilization was envisioned by the appearance of a clear zone on Sperber's plates, the diameter of clear zones along with the colony was measured using a slide calliper to calculate the phosphate solubilization index (PSI).

$$\text{PSI} = \frac{\text{Diameter of colony} + \text{Clear zone (mm)}}{\text{Colony diameter (mm)}}$$

Growth inhibition: Isolation of Sclerotium. Fungal-infected elephant foot yam [(*Amorphophallus paeoniifolius* (Dennst.) Nicolson)] was rinsed in tap water followed by sterile distilled water. The infected portion was excised and a few pieces were surface sterilized by rinsing in mercuric chloride (0.1%) followed by sterile distilled water. Surface-sterilized pieces were plated onto chloramphenicol-amended Potato Dextrose Agar (PDA) plates. The isolated fungal pathogen was purified, and its identity was confirmed as

Agroathelia rolfsii (Sacc.) Redhead & Mullineux and maintained on PDA slants until further studies.

Antagonistic activity. The antagonistic efficiency of *Trichoderma* isolates was assessed by dual culture technique against *Sclerotium* [Es-Soufi et al., 2020]. *Agroathelia rolfsii* and *Trichoderma* isolates were grown on PDA for up to seven days at laboratory temperature (28±2°C). Culture discs (5 mm) of *Agroathelia* from the colony periphery were aseptically transferred to PDA. The *Trichoderma* isolates were aseptically transferred into the same plate opposite to *A. rolfsii*. The plates were incubated at laboratory temperature (28±2°C) for seven days. Percent growth inhibition was calculated based on the diameter of colonies. Culture discs of *A. rolfsii* in plates without *Trichoderma* isolates served as a control.

(Where C, is the colony diameter of *A. rolfsii* without *Trichoderma* isolate; T, is the colony diameter of *A. rolfsii* along with *Trichoderma* isolate).

$$\text{Inhibition (\%)} = \frac{C - T}{C} \times 100$$

Cellulolytic Activity. Trichoderma isolates were inoculated onto mineral salt medium (K_2HPO_4, 1 g; $NaNO_3$, 0.5 g; $MgSO_4.7H_2O$, 0.5 g; $FeSO_4.7H_2O$, 0.02 g; KCl, 0.2 g; agar, 20 g; distilled water, 1 l; pH 7.5) supplemented with 0.05% (w/v) carboxymethyl cellulose (CMC) plates and incubated up to four days at laboratory temperature (28±2°C) [Demissie et al., 2024]. On the fourth day, the plates were flooded with an aqueous solution of Congo red (1%). The isolates showing a clear zone around the growth were considered positive for cellulase production.

Molecular Identification. Trichoderma isolate AMCC 3 was identified based on sequencing of the Internal Transcribed Spacer (ITS) region. The sequence thus obtained was analyzed by using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Morgulis et al., 2008]. The top five sequences of taxa belonging to the *Trichoderma* species, compatible with the isolate of this study, were obtained to construct the phylogenetic tree. Multiple sequence alignment of six sequences was established using ClustalW software, which was used to construct a phylogenetic tree through the Neighbour-Joining method [Saitou, Nei, 1987]. Statistical significance was evaluated with a bootstrapping of 500 repetitions [Felsenstein, 1985].

Data analysis. The difference in seed germination, seeding growth and growth inhibition of *A. rolfsii* by *Trichoderma* isolates against control were assessed using a t-test [StatSoft Inc, Version 8, 2008]. The same test was applied for phosphate solubilization and cellulase production between isolates.

RESULTS AND DISCUSSION

Trichoderma isolates obtained from the diverse rhizosphere habitats in the present study may be due to their varied metabolic capabilities and aggressive competitiveness against other microorganisms in lateritic loamy soils of southwest India. Four rhizosphere isolates of *Trichoderma* assessed for plant growth stimulation and pathogen inhibition showed diverse results.

Plant growth stimulation. A key component of the performance of crops is due to complex traits of seed vigour. Crop yield and resource use efficiency depend on successful plant growth in the field (the ability of seeds to germinate and establish seedlings rapidly across diverse environmental conditions) [Finch-Savage, Bassel, 2016]. Culture filtrates of all *Trichoderma* isolates in our study significantly enhanced the germination of seeds of mung bean ($p<0.05$), with the highest rate of germination by AMCC 3 (60 vs. 90%) (Fig. 1A). Culture filtrates of three isolates (AMCC 2-4) also significantly increased the radical growth of mung bean ($p<0.05$), with the highest radical length by AMCC 3 (87.2%) (Fig. 1B). Among four *Trichoderma* isolates, culture filtrate of AMCC 3 significantly enhanced root growth in *Amorphophallus paeoniifolius* (10 vs. 20 cm) ($p<0.05$), while corm density was also significantly increased by AMCC 2 (5 vs. 10) ($p<0.05$). Inayati et al. [2021] suggested that *Trichoderma virens* serve as a potential growth promoter and biocontrol agent against pathogens of mung bean. Isolates of *T. virens* (Tv3 and Tv4) showed increased height and biomass of mung bean [Inayati et al. 2021]. Okoth et al. [2011) reported that *Trichoderma* increases seed germination and promotes the growth of primary root length and root branching in maize and beans by inducing lateral root growth. In plants, auxins have been demonstrated to initiate lateral root growth by Casimiro et al. [2001]. *Trichoderma* virens (isolates Tv3 and Tv4) showed significantly high IAA synthase in leaves of mung bean [Inayati et al., 2021].

Contreras-Cornejo et al. [2009] showed that *Trichoderma* spp. produced indole-3-acetic acid (IAA) that promoted the lateral root formation in *Arabidopsis*

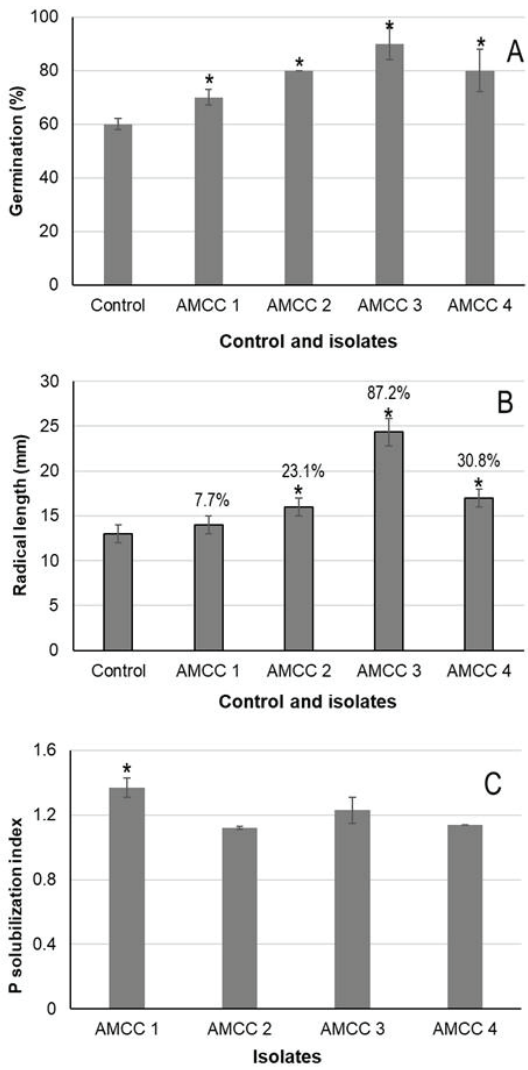

Figure 1. A, Influence of *Trichoderma* culture filtrate on seed germination of mung bean; B, Influence of *Trichoderma* culture filtrate on growth of radicle of mung bean; C, Phosphate solubilization index of *Trichoderma* isolates (n=3, mean±SD; t-test, *, $p<0.05$).

thaliana. It was similar to *in vitro* experiments carried out by Hexon et al. [2009] that promoted lateral roots in *Arabidopsis thaliana* by *Trichoderma* spp. by IAA. Inayati et al. [2021] demonstrated that *T. viriens* produce a high quantity of IAA on Czapek Dox agar. The leaf as well as tiller numbers have been found significantly higher in rice plants treated with *Trichoderma* spp. compared to control and NPK treatment [Doni et al., 2014]. Piotrowski and Volmer (2006) showed that the genomes of *Trichoderma* spp. contain many genes that encode nitrilases as compared with other fungi. Such nitrilases may have a role either in hydrolyzing β cyano l-alanine (a metabolite formed from cyanide release during the final step of ethylene biosynthesis) or in converting the plant metabolite indole 3 acetonitrile into IAA. In our study, the HPLC analysis of standard IAA (HiMedia Laboratories Private Limited, Mumbai, Maharashtra, India) and culture filtrate of AMCC 3 revealed the presence of a moderate amount of IAA (11.5 µg/ml) (Fig. 2A, B). The retention time of IAA of isolate AMCC 3 (13.926 min) was matched with standard IAA at 228 nm. According to Contreras-Cornejo et al. [2009], *T. virens* produced IAA, indole-3-acetaldehyde (IAAld) and indole-3-ethanol (IEt), which have played pivotal roles in plant growth and development. Substantial production of IAA by AMCC 3 might have resulted in increased germination as well as enhanced radical length of mung bean.

Phosphorus is the second most important macronutrient needed for the plants, next to nitrogen. Due to the scarcity of phosphorus in soluble form, it is reported that soluble phosphorus is a critical factor for the production of many crop systems [Xiao et al., 2011]. Some microbes are capable of dissolving insoluble forms of phosphorus and making it available for plant growth. *Trichoderma virens* showed high phosphate solubilizing activity in plate assays after six days [Inayati et al., 2021]. All the isolates of *Trichoderma* in our study showed phosphate solubilization in Sperber's medium. The PSI was significantly higher in AMCC 1 (1.37) ($p<0.05$) compared to other isolates (1.12-1.23) (Fig. 1C). The PSI represented by *Trichoderma* isolates in our study fairly matches with that of some strains of *T. virens* as reported by Inayati et al. [2021].

Growth inhibition. Trichoderma spp. possess antagonistic and mycoparasitic potential, hence reducing the disease severity [Viterbo, Horwitz, 2010]. With an intention to study the antagonistic property of the isolated *Trichoderma* isolates, pathogenic fungi *A. rolfsii* was isolated from the yam and cocultured with *Trichoderma* isolates (Fig. 3A). All the isolates significantly inhibited the growth of *A. rolfsii* (35-60%) ($p<0.05$), with the highest inhibition by AMCC 3 (60%) ($p<0.01$). Inbar et al. [1996] reported that *Trichoderma* parasitizes the *A. rolfsii* and controls its growth. Conidial suspensions of *Trichoderma* spp. prevented many diseases in sunflower and mug bean caused by *A. rolfsii* (e.g., damping off, root rot and seed rot) along with growth enhancement [Yaqub, Shahzad, 2008]. Siddiquee et al. [2012] demonstrated inhibitory effects of volatile and non-volatile antifungal compounds

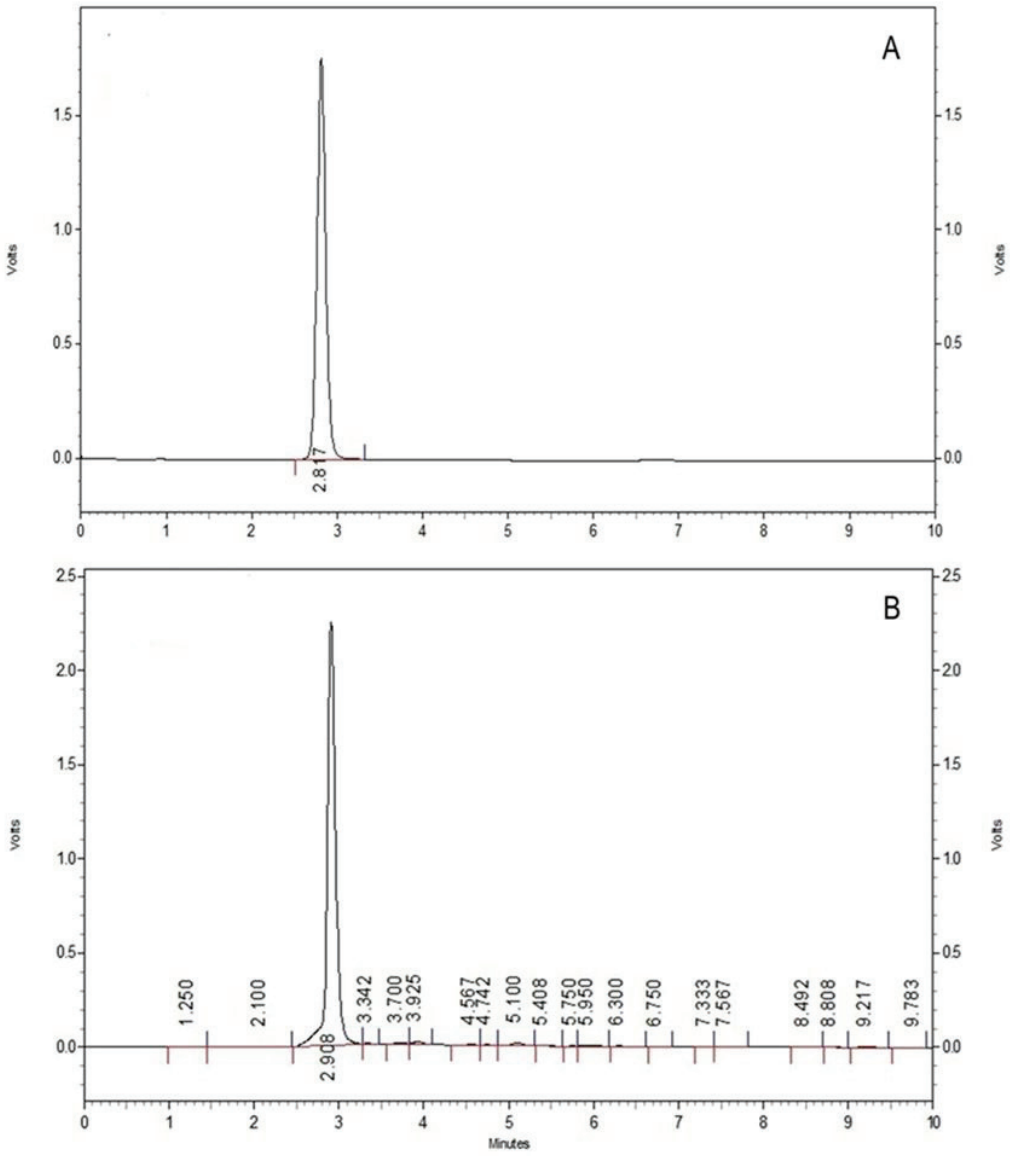

Figure 2. HPLC chromatogram: A, IAA profile of standard; B, IAA profile of AMCC 3.

produced by *Trichoderma*. *Harzianum*, *T. roseum* against *Ganoderma boninense*, which is causing stem rot in oil palm.

Culture filtrates of *Trichoderma* are known to possess several secondary metabolites [e.g., 6-nonylene alcohol, massoilactone, methyl-cyclopentane, methyl-cyclohexane, N-methyl pyrollidine, dermadin, ketotriol, koningin-A, 3-methyl-heptadecanol, 2-methyl heptadecanol, palmitic acid, 3-(2'-hydroxypropyl)-4-(hexa-2'-4-dineyl)-2-(5H) furanone and 3-(propenone)4-(hexa-2'-4'-dineyl)-2-(5H)-furanone] [Dubey et al., 2011). Dubey et al. [2011] also demonstrated that secondary metabolites extracted from Trichoderma inhibited plant pathogenic fungi (e.g., *Fusarium oxysporum* and *Rhizoctonia bataticola*). Similarly, Vinale et al. [2009] evaluated production and antibiotics produced by *T. harzianum* strains in vitro and found several major compounds synthesized against many pathogenic fungi and they showed different levels of antibiotic activity.

Mycoparasitism of *Trichoderma* spp. is a complex phenomenon, which recognizes signals from the pathogenic fungus, coils around host hyphae and penetrates. The lytic enzymes, such as chitinases, glucanases and proteases of *Trichoderma*, degrade the host cell, leading to the death of the pathogen [Steyaert et. al., 2004; Sharma et al., 2012]. Singh et al. [2018] found that *Trichoderma* releases cellulases that degrade

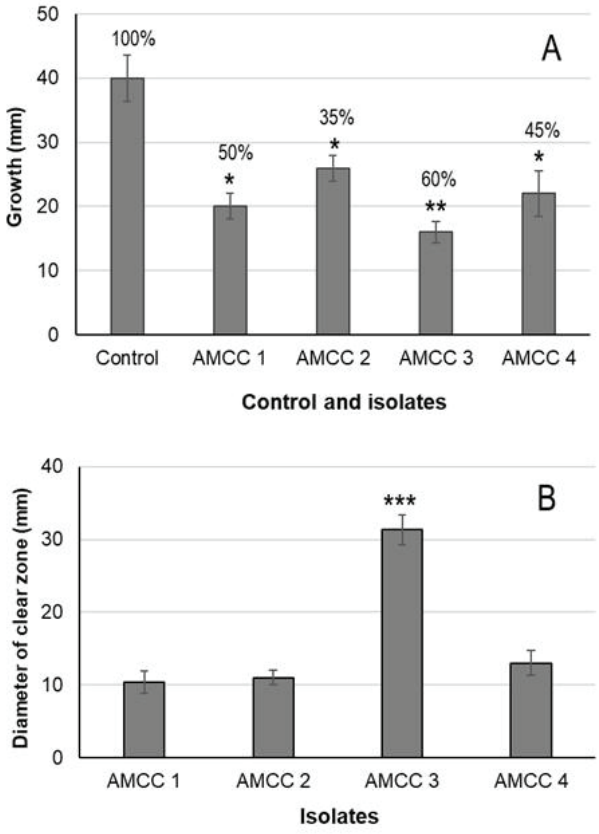

Figure 3. A, Inhibition of *Agroathelia rolfsii* colony growth under the influence of Trichoderma isolates (n=3, mean±SD; t-test, *, $p<0.05$, **, $p<0.01$); B, Diameter of carboxymethyl cellulose digestion by *Trichoderma* isolates (n=3, mean±SD; t-test, ***, $p<0.001$).

cellulose and it also enhances the decomposition of organic matter. It has been reported that the cell wall disruption of pathogenic fungi (e.g., *Pythium* spp.) takes place by production of cellulase [Zerillo et al., 2013]. Nevalainen and Penttilä [1995] demonstrated that cellulases hydrolyze β-1,4 glucans and serve as the most effective weapons for biological control of plant diseases. Hyder et al. [2017] reported cellulose activity by *Trichoderma* spp. All the *Trichoderma* isolates produced a clear zone upon adding Congo red dye solution around the colony grown on CMC medium, indicating production of cellulase (Fig. 3B). Isolate AMCC 3 produced a significantly higher clear zone compared with other isolates (31.33 vs. 10.33-13 mm) ($p<0.001$). Mostly, *Trichoderma* isolates in the present study employ cellulase to control pathogens and decompose organic matter to meet its nutritional requirements.

Phylogeny of AMCC 3. As *Trichoderma* isolate AMCC 3 emerged as almost the best-performing isolate for seed germination, enhancing radical length, growth inhibition of *A. rolfsii* and high cellulase production, hence it was subjected to ITS1 and ITS4 sequence analysis [Samuels et al., 2009]. A phylogenetic tree was constructed for AMCC 3 based on its ITS sequence with the ITS regions of six *Trichoderma* isolates obtained from GenBank (http:/ncbi.nih.gov) as a reference. Based on the BLASTN search and phylogeny analysis, isolate AMBC-3 was matched best with *Trichoderma erinaceum* Bissett, C.P. Kubieck & Szakács (Fig. 4). In fact, *Trichoderma erinaceum* is already well known for

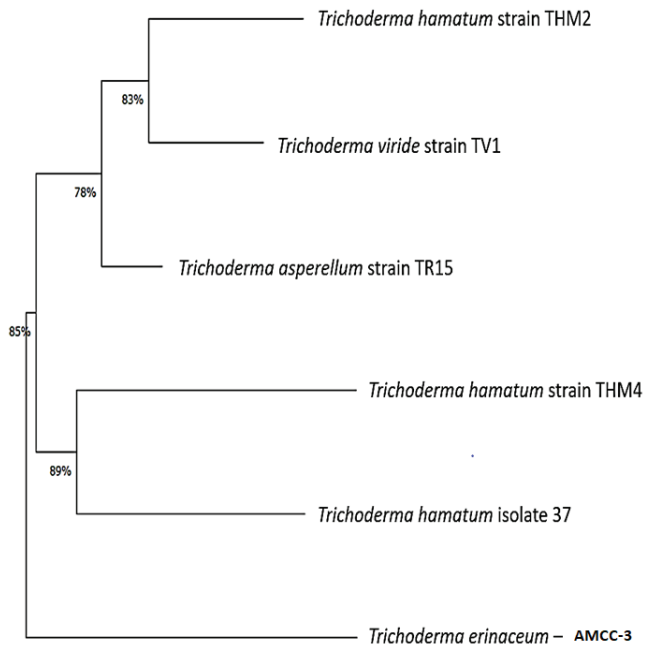

Figure 4. Phylogenetic tree of *Trichoderma* isolate AMBC 3 matching with *Trichoderma erinaceum*.

the protection of tomato plants (*Solanum lycopersicum* L.) against the vascular wild pathogen *Fusarium oxysporum* due to the expression of chitinases and glucanases [Aamir et al., 2019].

CONCLUSION

Four *Trichoderma* isolates derived from the rhizosphere of four horticulture plant species in southwest India revealed plant growth promotion, phosphate solubilization, growth inhibition of phytopathogenic fungi Agroathelia rolfsii and cellulolytic activity. The isolate AMCC 3 is phylogenetically corresponding with *Trichoderma erinaceum*. It has shown the highest seed germination as well as radical length in mung bean. It

also showed good phosphate solubilizing activity, IAA production, the highest inhibition against *A. rolfsii* and the highest cellulolytic potential among the isolates. Further precise evaluation of *T. erinaceum* (AMCC 3) needs to adapt as a potential plant growth stimulant and biocontrol agent in the native lateritic loamy soils of southwest India.

ACKNOWLEDGEMENTS

The authors are thankful to Alva's Education Foundation, Moodbidri, for supporting this study. We thank Dr. Mahadevakumar, Scientist, Botanical Survey of India, Andaman and Nicobar Regional Centre, Port Blair, India, for technical support.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest to publish this article.

REFERENCE

Aamir M., Kashyap S.P., Zehra A., Dubey M.K., Singh V.K. et al. (2019) *Trichoderma erinaceum* bio-priming modulates the WRKYs defense programming in tomato against the *Fusarium oxysporum* f. sp. *lycopersici* (Fol) challenged condition. *Front. Plant Sci*., 10: 911. https://doi.org/10.3389/fpls.2019.00911

Abdou K.H.A. (2018) Epidemiology of pesticides in developing countries. *Adv. Clin. Toxicol*., 3(1). https://doi.org/10.23880/act-16000128

Amina M., Bensoltane A., Benidire L., Oufdou K. (2017) Plant growth-promotion and IAA secretion with P*seudomonas fluorescens* and *Pseudomonas putida*. *Res. Rev. J. Bot. Sci*., 6(2): 16-22.

Asghar W., Craven K.D., Kataoka R., Mahmood A., Asghar N. et al. (2024) The application of *Trichoderma* spp., an old but new useful fungus, in sustainable soil health intensification: A comprehensive strategy for addressing challenges. *Pl. Stress*, 12(100455): 100455. https://doi.org/10.1016/j.stress.2024.100455

Casimiro I., Marchant A., Bhalerao R.P., Beeckman T., Dhooge S. et al. (2001) Auxin transport promotes *Arabidopsis* lateral root initiation. *The Plant Cell*, 13(4): 843–852. https://doi.org/10.1105/tpc.13.4.843

Chaube H.S., Mishra D.S., Varshney S., Singh U.S. (2002) Biological control of plant pathogens by fungal antagonistics: Historical background, present status and future prospects. *Ann. Rev. Pl. Pathol*., 2: 1-42.

Contreras-Cornejo H.A., Macías-Rodríguez L., Cortés-Penagos C., López-Bucio J. (2009) *Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in Arabidopsis. *Pl. Physiol*., 149(3): 1579-1592. https://doi.org/10.1104/pp.108.130369

Damalas C., Koutroubas S. (2016) Farmers' exposure to pesticides: Toxicity types and ways of prevention. *Toxics*, 4(1): 1. https://doi.org/10.3390/toxics4010001

Demissie M.S., Legesse N.H., Tesema A.A. (2024) Isolation and characterization of cellulase producing bacteria from forest, cow dung, Dashen brewery and agro-industrial waste. *PloS one*, 19(4): e0301607. https://doi.org/10.1371/journal.pone.0301607

Doni F., Isahak A., Zain C.R.C.M., Yusoff W.M. (2014) Physiological and growth response of rice plants (*Oryza sativa* L.) to *Trichoderma* spp. inoculants. *AMB Express*, 4(1). https://doi.org/10.1186/s13568-014-0045-8

Druzhinina I.S., Seidl-Seiboth V., Herrera-Estrella A., Horwitz B.A., Kenerley C.M. et al. (2011) *Trichoderma*: The genomics of opportunistic success. *Nat. Rev*., 9: 749-759.

Dubey S.C., Tripathi A., Dureja P., Grover A. (2011) Characterization of secondary metabolites and enzymes produced by *Trichoderma* species and their efficacy against plant pathogenic fungi. *Ind. J. Agric. Sci*., 81(5): 455-461.

Elad Y. (2000) *Trichoderma harzianum* T39 preparation for biocontrol of plant diseases-control of *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Cladosporium fulvum*. *Biocon. Sci. Technol*., 10(4): 499-507.

Es-Soufi R., Tahiri H., Azaroual L., Oualkadi A.E., Martin P. et al. (2020) In vitro antagonistic activity of *Trichoderma harzianum* and *Bacillus amyloliquefaciens* against *Colletotrichum acutatum*. *Adv. Microbiol*., 10(03): 82-94. https://doi.org/10.4236/aim.2020.103008

Felsenstein J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39: 783-791.

Finch-Savage W.E., Bassel G.W. (2016) Seed vigour and crop establishment: extending performance beyond adaptation. *J. Exp. Bot*., 67(3): 567-591. https://doi.org/10.1093/jxb/erv490

Garuba T., Abdulrahaman A.A., Olahan G.S., Abdulkareem K.A., Amadi J.E. (2015) Effects of fungal filtrates on seed germination and leaf anatomy of maize seedlings (*Zea mays* L., Poaceae). *J. Appl. Sci. Environ. Manag*., 18(4): 662. https://

doi.org/10.4314/jasem.v18i4.15

Ha T.N. (2010) Using *Trichoderma* species for biological control of plant pathogens in Vietnam. *J. ISSAAS*., 16(1): 17-21.

Harman G.E. (2011) *Trichoderma* - not just for biocontrol anymore. Phytoparasitica: *Israel J. Pl. Prot. Sci*., 39(2): 103-108. https://doi.org/10.1007/s12600-011-0151-y

He D.-C., He M.-H., Amalin D.M., Liu W., Alvindia D.G., Zhan J. (2021) Biological control of plant diseases: An evolutionary and Eco-economic consideration. *Pathogens*, 10(10): 1311. https://doi.org/10.3390/pathogens10101311

Hexon A.C., Lourdes M.R., Carlos C.P., Jose L.B. (2009) *Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in *Arabidopsis. Pl. Physiol*., 149: 1579-1592.

Hyder S., Inam-ul-Haq M., Bibi S., Malik A.H., Ghuffar S., Iqbal S. (2017) Novel potential of *Trichoderma* spp. as biocontrol agent. *J. Entomol. Zool. Stud*., 5(4): 214-222

Inayati A., Setyoweti L., Aini L.Q., Yusnawan E. (2021) Plant growth promoter produced by T*richoderma virens* and its effect on mungbean (*Vigna radiata* (L.) Wilczek) seedling. *Earth Environ. Sci*., 803: 012013. https://doi.org/10.1088/1755-1315/803/1/012013

Inbar J., Menendez A., Chet I. (1996) Hyphal interaction between *Trichoderma harzianum* and *Sclerotinia sclerotiorum* and its role in biological control. *Soil Biol. Biochem*., 28: 757-763.

Jambhulkar P.P., Singh B., Raja M., Ismaiel A., Lakshman D.K. et al. (2024) Genetic diversity and antagonistic properties of *Trichoderma* strains from the crop rhizospheres in southern Rajasthan, India. *Sci. Rep*., 14(1). https://doi.org/10.1038/s41598-024-58302-5

Kumar S. (2013) *Trichoderma*: A biological control weapon for managing plant diseases and promoting sustainability. *Int. J. Agric. Sci. Veter. Med*., 1: 106-121.

Morgulis A., Coulouris G., Raytselis Y., Madden T.L., Agarwala R., Schäffer A.A. (2008) Database indexing for production MegaBLAST searches. *Bioinformatics*, 24(16); 1757-1764.

Moser R., Pertot I., Elad Y., Raffaelli R. (2008) Farmers' attitudes toward the use of biocontrol agents in IPM strawberry production in three countries. *Biol. Cont*., 47(2): 125-132. https://doi.org/10.1016/j.biocontrol.2008.07.012

Naher L., Syawani N., Amieza N., Kamarudin A.B., Karim S.M.R. (2019) *Trichoderma* species diversity in rhizosphere soils and potential antagonism with *Fusarium oxysporum*. *Biosci. J*., 13-26. https://doi.org/10.14393/bj-v35n1a2019-41605

Naidu R., Biswas B., Willett I.R., Cribb J., Singh B.K. et al. (2021) Chemical pollution: A growing peril and potential catastrophic risk to humanity. *Environ. Int*., 156: 106616. https://doi.org/10.1016/j.envint.2021.106616

Nakkeeran S., Kavitha K., Mathiyazhagan S., Fernando W.G.D., Chandrasekar G., Renukadevi P. (2004) Induced systemic resistance and plant growth promotion by *Pseudomonas chlororaphis* strain PA-23 and *Bacillus subtilis* strain CBE4 against rhizome rot of turmeric (*Curcuma longa* L.). *Can. J. Pl. Pathol*., 26: 417-418.

Natsiopoulos D., Tziolias A., Lagogiannis I., Mantzoukas S., Eliopoulos P.A. (2022) Growth-promoting and protective effect of *Trichoderma atrobrunneum* and *T. simmonsii* on tomato against soil-borne fungal pathogens. *Crops*, 2: 202-217. https://doi.org/10.3390/crops2030015

Nevalainen H., Penttilä M. (1995) Molecular biology of cellulolytic fungi. In: The Mycota II. Genetics and biotechnology, Kuck U. (Ed.), Springer Verlag, Berlin, pp 303-319.

Okoth S.A., Otadoh J.A., Ochanda J.O. (2011) Improved seedling emergence and growth of maize and beans by *Trichoderma harzianum*. *Trop. Subtrop. Agroecosys*., 7: 65-71.

Pandit M.A., Kumar J., Gulati S., Bhandari N., Mehta P. et al. (2022) Major biological control strategies for plant pathogens. *Pathogens*, 11(2): 273. https://doi.org/10.3390/pathogens11020273

Piotrowski M., Volmer J.J. (2006) Cyanide metabolism in higher plants: cyanoalanin-e hydratase is a NIT4 homolog. *Pl. Mol. Biol*., 61: 111-122.

Rahman A., Begum M.F., Rahman M., Bari M.A., Illias G.N.M., Alam M.F. (2011) Isolation and identification of *Trichoderma* species from different habitats and their use for bioconversion of solid waste. *Turkish J. Biol*., 35(2): 183-194. https://doi.org/10.3906/biy-0905-8

Saitou N., Nei M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol*., 4(4): 406-425.

Saldaña-Mendoza S.A., Pacios-Michelena S., Palacios-Ponce A.S., Chavez-Gonzalez M.L., Aguilar C.N. (2023) *Trichoderma* as a biological control agent: mechanisms of action, benefits for crops and development of formulations. *World*

J. Microbiol. Biotechnol., 39: 269. https://doi.org/10.1007/s11274-023-03695-0

Samuels G.J., Chaverri P., Farr D.F., McCray E.B. (2009) *Trichoderma* Online. Systematic mycology and microbiology laboratory, ARS, USDA; http://nt.ars-grin.gov/taxadescriptions/keys/TrichodermaIndex.cfm

Sharma B.L., Singh S.P., Sharma M.L. (2012) Biodegradation of crop residues by *Trichoderma* species vis-à vis nutrient quality of the prepared compost. *Sugar Tech*., 14(2): 174-180. https://doi.org/10.1007/s12355-011-0125-x

Siddiquee S., Cheong B.E., Taslima K., Kausar H., Hasan M.M. (2012) Separation and identification of volatile compounds from liquid cultures of *Trichoderma harzianum* by GC-MS using three different capillary columns. *J. Chromatogr. Sci*., 50(4): 358-367.

Singh A, Shukla N., Kabadwal B.C., Tewari A.K., Kumar J. (2018) Review on plant-*Trichoderma*-pathogen interaction. *Int. J. Curr. Microbiol. Appl. Sci*., 7(2): 2382-2397. https://doi.org/10.20546/ijcmas.2018.702.291

Sood M., Kapoor D., Kumar V., Sheteiwy M.S., Ramakrishnan M. et al. (2020) *Trichoderma*: The "secrets" of a multitalented biocontrol agent. *Plants*, 9(6): 762. https://doi.org/10.3390/plants9060762

Sperber J.I. (1957) Solution of mineral phosphates by soil bacteria. *Nature*, 180(4593): 994-995. https://doi.org/10.1038/180994a0

StatSoft Inc. (2008) Statistica Version # 8. Tulsa, Oklahoma, USA.

Stewart A., Hill R. (2014) Applications of *Trichoderma* in plant growth promotion. In: Biotechnology and biology of *Trichoderma*, Gupta V.K., Schmoll, M., Herrera-Estrella A., Updhyay R.S., Druzhinina I, Touhy M.G. (Eds.), Elsevier, pp 415-428. https://doi.org/10.1016/B978-0-444-59576-8.00031-X

Steyaert J.M., Stewart A., Jaspers M.V., Carpenter M., Ridgway H.J. (2004) Co-expression of two genes, a chitinase (*chit42*) and proteinase (*prb1*), implicated in mycoparasitism by T*richoderma hamatum*. *Mycologia*, 96: 1245-1252.

Tyśkiewicz R., Nowak A., Ozimek E., Jaroszuk-Ściseł J. (2022) *Trichoderma*: The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. *Int. J. Mol. Sci*., 23(4): 2329. https://doi.org/10.3390/ijms23042329

United Kingdom Food Security Report (2021) Official Statistics United Kingdom Food Security Report 2021: Theme 1: Global Food Availability (Updated 22 October 2024).

Vinale F., Flematti G., Sivasithamparam K., Lorito M., Marra R. et al. (2009) Harzianic acid, an antifungal and plant growth promoting metabolite from *Trichoderma harzianum. J. Nat. Prod*., 72(11): 2032-2035. https://doi.org/10.1021/np900548p

Vinale F., Sivasithamparam K., Ghisalberti E.L., Marra R., Barbetti M.J. et al. (2008) A novel role for *Trichoderma* secondary metabolites in the interactions with plants. *Physiol. Mol. Pl. Pathol*., 72(1-3): 80-86. https://doi.org/10.1016/j.pmpp.2008.05.005

Viterbo A., Horwitz B.A. (2010) Mycoparasitism. In: Cellular and molecular biology of filamentous fungi, Volume 42, Borkovich K.A., Ebbole D.J. (Eds.), American Society for Microbiology, AAM Press, Wiley, pp 676-693.

Xiao C.Q., Chi R.A., Li W.S., Zheng Y. (2011). Biosolubilization of phosphorus from rock phosphate by moderately thermophilic and mesophilic bacteria. *Min. Eng*., 24(8), 956-958. https://doi.org/10.1016/j.mineng.2011.01.008

Yaqub F., Shahzad, S. (2008) Effect of seed pelleting with *Trichoderma* spp., and *Gliocladium virens* on growth and colonization of roots of sunflower and mugbean by *Sclerotium rolfsii*. *Pak. J. Bot*., 40: 947-963.

Zerillo M.M., Adhikari B.N., Hamilton J.P., Buell C.R., Lévesque C.A., Tisserat N. (2013) Carbohydrate-active enzymes in *Pythium* and their role in plant cell wall and storage polysaccharide degradation. *PloS One*, 8(9): e72572. https://doi.org/10.1371/journal.pone.0072572

Hindistanın cənub-qərbindən *Trichoderma* cinsinə aid dörd rizosfer nümunəsinin bitki böyüməsinin təşviqi və antipatogen göbələk fəallığı

Hosamane Kavya
Biotexnologiya üzrə diplomdan sonrakı təhsil departamenti, Alva Muxtar Kolleci, Moodbidri, Karnataka, Hindistan

Əli Cavad Akki
Biotexnologiya şöbəsi, Tətbiqi Elm və Texnologiya Məktəbi, Bijapur Lingayat Rayon Təhsil Assosiasiyası (Universitet hesab olunur), Vijayapura, Karnataka, Hindistan

Raghavendra Rao Badkillaya
Biotexnologiya üzrə diplomdan sonrakı təhsil departamenti, Alva Muxtar Kolleci, Moodbidri, Karnataka, Hindistan

Santhoş Uilson Goveas
Biotexnologiya şöbəsi, St. Aloysius (Universitet hesab olunur), Mangalore, Karnataka, Hindistan

Kandikere Ramaiah Şridhar
Bioelmlər Departamenti, Manqalor Universiteti, Manqalaganqotri, Manqalor, Karnataka, Hindistan

Trichoderma bitki artımını stimullaşdırmaq potensialına malikdir və fitopatogen göbələklərin antaqonistidir. Dörd *Trichoderma* ştammı banan (*Musa acuminata*), muskat (*Myristica fragrans*), bibər (*Piper nigrum*) və Hindistan tamarindası (*Tamarindus indica*) (AMCC 1-4) rizosferlərindən ayrılmışdır. Onların kultura ekstraktları maş paxlasında (*Vigna radiata*) toxumların cücərməsi, radikal artım və nəsli kəsilməkdə olan Yeməli fil ayağı (*Amorphophallus paeoniifolius*) bitkisinin kök yumrunun inkişafı üçün qiymətləndirilmişdir. Bütün *Trichoderma* kulturalarının filtratı toxumların cücərməsini əhəmiyyətli dərəcədə artırmış, həmçinin maş paxlasının radikal böyüməsini artıraraq, AMCC 3 (90%) ilə yüksək cücərmə və inkişaf nümayiş etdirmişdir. AMCC 3 həmçinin Yeməli fil ayağı bitkisinin kökünün böyüməsini sürətləndirmiş (20 ilə müqayisədə 10 sm), kökyümurusunun sıxlığı AMCC 2 (10 ilə müqayisədə 5) kimi artmışdır. Yüksək göstəricili maye xromatoqrafiyası (HPLC) ilə AMTC 3-ün kultura filtratının təhlili indol-3-sirkə turşusunun (IAA) (11.5 µg/ml) mövcudluğunu göstərib. *Trichoderma* cinsinin bütün nümunələri AMCC 1 (1.4) ilə ən yüksək həll olunma indeksi ilə fosfatları həll etmə qabiliyyətinə malikdir. Bütün *Trichoderma* nümunələrinin kultura filtratları fitopatogen *Agroathelia rolfsii* növünün *in vitro* artımını əhəmiyyətli dərəcədə inhibə edir, AMCC 3 (60%) ilə ən yüksək inhibə müşahidə edilmişdir. *Trichoderma* izolatlarını karboksimetilselüloz (CMC) aqarda dörd günədək becərdikdə, bütün nümunə koloniyalarının ətrafında onların sellülaz istehsal qabiliyyətini təsdiqləyən aydın zona görünür və ən yüksək nəticə AMCC 3 (31.3 mm) ilə əldə edilmişdir. AMCC 3-ün daxili transkripsiya sahəsi (ITS) geni ardıcıllaşdırılıb və BlastN proqramından istifadə edərək NCBI verilənlər bazası ilə müqayisə edilib. AMCC 3-ün DNT ardıcıllığı *Trichoderma erinaceum* növünün ardıcıllığına uyğundur. Bu tədqiqatda dörd bağçılıq bitkisi növünün rizosferindən dörd *Trichoderma* nümunəsinin bitki inkişafını sürətləndirdiyi, həmçinin *T. erinaceum* (AMCC 3) növünün fitopatogen *Agroathelia rolfsii* növünün böyüməsinə yüksək effektivliklə maneə törətdiyi göstərilmişdir. Növ Hindistanın cənub-qərbindəki laterit gilli torpaqlarda göbələk fitopatogenlərinə qarşı bitki inkişafının stimulyatoru və biopestisidi kimi istifadə edilə bilər.

Açar sözlər: *sellülolitik aktivlik, inkişafın inhibəsi, indol sirkə turşusu, fosfatı həll etmə qabiliyyəti, radikal inkişaf, Trichoderma erinaceum*

Стимуляция роста растений и противопатогенная грибковая активность четырех ризосферных изолятов *Trichoderma* с юго-запада Индии

Хосамане Кавья
Кафедра последипломного образования в области биотехнологии, Автономный колледж Альвы, Мудбидри, Карнатака, Индия

Али Джавад Акки
Кафедра биотехнологии, Школа прикладных наук и технологий, Образовательная ассоциация округа Биджапур-Лингаят (считается университетом), Виджаяпура, Карнатака, Индия

Рагхавендра Рао Бадкиллая
Отделение последипломного образования в области биотехнологии, Автономный колледж Альвы, Мудбидри, Карнатака, Индия

Сантош Уилсон Говеас
Кафедра биотехнологии, St. Aloysius (считается университетом), Мангалор, Карнатака, Индия

Кандикере Рамайя Шридхар
Департамент биологических наук, Университет Мангалора, Мангалаганготри, Мангалор, Карнатака, Индия

Trichoderma обладает потенциалом стимулировать рост растений и является антагонистом фитопатогенных грибов. Четыре штамма *Trichoderma* были выделены из ризосфер банана (*Musa acuminata*), мускатного ореха (*Myristica fragrans*), перца (*Piper nigrum*) и тамаринда (*Tamarindus indica*) (AMCC 1-4). Экстракты их культур оценивались на предмет прорастания семян, радикального роста маша (*Vigna radiata*), а также развития корней и клубнелуковиц у находящегося под угрозой исчезновения вида ямса пеонфолийского (*Amorphophallus paeoniifolius*). Фильтрат культуры всех изолятов *Trichoderma* значительно увеличил всхожесть семян, а также усилил прикорневой рост маша (*Vigna radiata*), при этом наибольшую всхожесть и прикорневой рост наблюдали у AMCC 3 (90%). AMCC 3 также усилил рост корней ямса (10 против 20 см), в то время

как плотность клубнелуковицы увеличилась на AMCC 2 (5 против 10). Анализ фильтрата культуры AMCC 3 методом высокоэффективной жидкостной хроматографии (ВЭЖХ) показал наличие индолил-3-уксусной кислоты (ИУК) (11.5 µg/ml). Все изоляты *Trichoderma* обладают фосфатной солюбилизацией, с самым высоким индексом солюбилизации у AMCC 1 (1.4). Фильтраты культур всех изолятов *Trichoderma* показали значительное подавление роста in vitro фитопатогена *Agroathelia rolfsii*, с самым высоким подавлением у AMCC 3 (60%). При культивировании изолятов *Trichoderma* на агаре с карбоксиметилцеллюлозой (КМЦ) в течение четырех дней все изоляты показали чистую зону вокруг колоний, что подтверждает их способность продуцировать целлюлазу, при этом наибольшая продукция была получена у AMCC 3 (31.3 мм). Ген внутреннего транскрибируемого спейсера (ITS) из AMCC 3 был секвенирован и сравнен с базой данных NCBI с помощью программы BlastN. Последовательность ДНК AMCC 3 соответствует последовательности ДНК *Trichoderma erinaceum*. В настоящем исследовании показано, что четыре изолята *Trichoderma* из ризосферы четырех видов садовых растений обладают способностью стимулировать рост растений, а также подавлять рост фитопатогена *Agroathelia rolfsii* с высокой эффективностью в отношении *T. erinaceum* (AMCC 3). Его можно использовать в качестве стимулятора роста растений и биопестицида против грибковых фитопатогенов в латеритных суглинистых почвах юго-западной Индии.

Ключевые слова: *целлюлозолитическая активность, ингибирование роста, индолилуксусная кислота, растворение фосфата, радикальный рост, Trichoderma erinaceum*