Manipal University Jaipur's Commitment to Empowering Local Farmers and Food Producers through Food Security Knowledge

Manipal University Jaipur (MUJ) is committed to supporting food security initiatives and promoting sustainable agricultural practices within the local community. As part of this mission, MUJ actively collaborates with local farmers and food producers, offering access to essential food security knowledge, resources, and training. By equipping these stakeholders with the tools and insights needed for sustainable farming, MUJ aims to contribute to the prosperity and resilience of the local agricultural community.

MUJ regularly conducts workshops, training sessions, and informational seminars focused on sustainable agriculture, food security, and innovative farming techniques:

These workshops cover a wide range of topics, such as sustainable farming methods, crop diversification, organic farming practices, and effective water management techniques. MUJ partners with agricultural experts, faculty, and students to provide hands-on training and practical knowledge to local farmers and food producers.

MUJ organizes seminars that explore food security concepts, including ways to optimize crop yields, reduce food waste, and improve storage and distribution practices. These seminars help farmers and food producers understand the various aspects of food security, empowering them to make decisions that enhance food availability and quality.

MUJ hosts field days where local farmers and food producers can observe and participate in demonstrations on sustainable farming practices. These events provide a platform for farmers to learn from experts and share their experiences with peers, fostering a sense of community and shared learning.

MUJ's training programs include sessions on soil testing, nutrient management, and composting techniques. Farmers learn how to maintain soil fertility through natural and sustainable practices, reducing their reliance on chemical fertilizers while improving crop productivity. Given the importance of water in agriculture, MUJ provides training on efficient irrigation methods, rainwater harvesting, and other water conservation strategies. This knowledge is crucial for local farmers who face challenges related to water scarcity, enabling them to use water resources more efficiently. MUJ educates farmers on integrated pest management (IPM) practices, which focus on environmentally friendly approaches to pest control. These methods help farmers reduce the use of harmful pesticides, protect local ecosystems, and produce safer, healthier food.

MUJ faculty and students engage in research initiatives that explore topics such as crop resilience, soil regeneration, and climate-adaptive farming. By partnering with local farmers, MUJ can test and refine new techniques that are tailored to the specific environmental conditions and needs of the region. MUJ conducts pilot programs that introduce and evaluate

sustainable farming practices within the local farming community. These programs allow farmers to experiment with new approaches, such as permaculture or agroforestry, with guidance from MUJ experts. MUJ facilitates the transfer of agricultural technologies, such as low-cost drip irrigation systems, mobile apps for farm management, and bio-fertilizers. By making these technologies accessible to local farmers, MUJ helps them adopt modern methods that improve efficiency and yield.

To enhance its impact, MUJ fosters strong community connections and support networks for local farmers and food producers:

MUJ provides advisory services to farmers, offering guidance on topics such as crop planning, pest management, and market trends. These services are available year-round, ensuring that farmers can access support when they need it most. MUJ collaborates with local NGOs, agricultural cooperatives, and government agencies to deliver comprehensive support to farmers. These partnerships facilitate the sharing of resources, knowledge, and expertise, enabling a more coordinated approach to addressing food security challenges.

Manipal University Jaipur is dedicated to supporting local farmers and food producers in their efforts to achieve food security and sustainability. Through workshops, research collaborations, resource management training, and community partnerships, MUJ aims to empower these essential members of our community with the knowledge and tools needed to thrive. By promoting sustainable agriculture and enhancing food security, MUJ not only strengthens the local agricultural sector but also contributes to the well-being of the entire region. In supporting local farmers, MUJ reaffirms its commitment to fostering a resilient, sustainable, and prosperous future for all.

Skills to local farmers and food producers

Introduction and Demonstration given by Expert, Ms. Geeta Ahluwalia

Demonstration of Kokedama given by Expert, Ms. Geeta Ahluwalia

EVENT REPORT

MANIPAL UNIVERSITY
JAIPUR

FACULTY OF DESIGN

HERITAGE CLUB

School of Architecture and Design

FOOD WALK

Walled City, Jaipur

18th FEBRUARY 2023

Contents

1. Introduction of the Event:

Heritage Club, School of Architecture and Design conducted a Food walk through a well curated route in the walled city of Jaipur for the students of MUJ, where students got to explore the Heritage City of Jaipur through its tasty delicacies while also enjoying the religious vibe in the walled city on the occasion of Mahashivratri.

2. Objective of the Event:

The curated route of Food Walk took the participants to the pink city in order to help them appreciate and admire the following –

- The scrumptious local delicacies along with their specific history.
- The streets featuring continuous small scaled shops and local vendors that thrive upon the city's heart.
- The built heritage of walled city, as the route also covered prominent architectural structures such as Hawa Mahal, Tripolia Gate and Tarkeshwar temple(one of the most prominent shiva temple in Jaipur) etc.

3. Beneficiaries of the Event:

- Students from all faculties of MUJ.
- Faculty members of MUJ

4. Brief Description of the event:

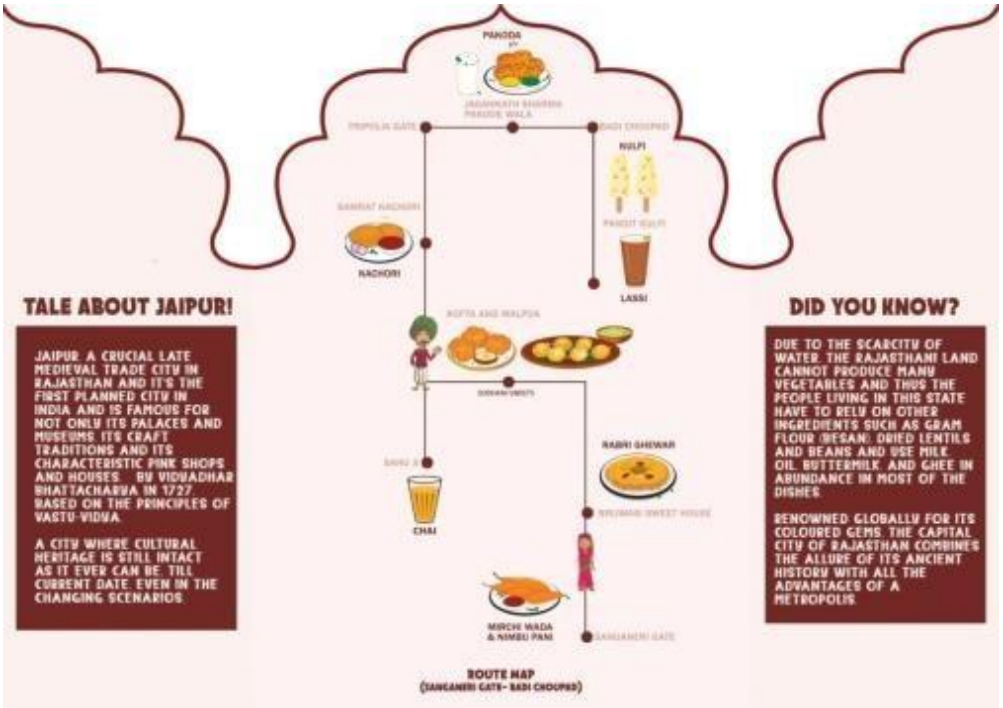
Heritage Club (School of Architecture and Design) conducted a Food Walk in the Walled City of Jaipur to acquaint the student fraternity of MUJ with the food delicacies and heritage beauty of Jaipur, on 18th February 2023. The food walk included several food items which offered different tastes of local Rajasthani cuisines where students could appreciate and admire the taste.

This Food Walk was the third physical event of the Heritage Club, but first of its kind ever. The walk began from Sanganeri Gate and terminated at Zaleb Chowk (route details as per brochure on the following page). Besides briefing about the history of the traditional bazaars, participants were also enlightened about the legacy of diverse cuisines by the enthusiastic food vendors themselves who showed utmost hospitality to the group of students and faculties.

The food menu provided a variety of items ranging from local drinks like nimbu pani to snacks like mirchi bada, kachoris & pakoras to sweets like ghewar and kulfi. The portions of all food items were specially made uniquely after requesting the vendors so that the portions then become easily consumable by a single person. Hence, everyone got an opportunity to taste all items (and the walk made it easy to digest them and create an appetite for the next item).

The walk ended with a positive feedback and contentment by all the participants. Mementos (key chains) created in-house by the club and school were distributed to everyone as a token of memory and gratitude. E-certificates were also given to all participants and volunteers of the event.

5. Brochure of the event



6. Photographs of the event.

Picture 1- Participants of the Food Walk

Picture 2-Sodhani Sweets (2nd stop)
Food vendors presenting the importance and making of malpua and aloo vada along with the narrative on when the shop was opened.

Picture 3- Sahoo Restaurant (3rdstop)
Participants having a break with Tea.

Picture 4 – Jagannath Pakode Wale (4th stop).

Picture 5- Pandit Kulfi (last stop).

7. Attendance of the Event:

ID	Name2	Registration Number	Course & Branch	h/d	Contact	Signature
1	Divyesh Shankla	210501003	Barch	Day scholar	9894699992	
2	Mansi	200501024	B.arch	Day scholar	7877991098	
3	Ria Rattan Kotwal	210501028	B.Arch	Hosteler	7406524738	
4	Jai	200901001	BBA	Hosteler	969093693	
5	Sonali	211002041	Bio science	Hosteler	8770273605	
6	Sanchoita	200606015	B.ARCH	Hosteler	8408803378	
7	Hrishita Kesarwani	229303266	Cse ai ml	Hosteler	9922490410	
8	Anjali Adhikari	210501025	B Arch	Hosteler	9733182131	
9	Priya Agarwal	211002006	Bsc biotechnology (biosciences)	Hosteler	7061587976	
10	Bhavesh Khemka	210501009	Architecture	Day scholar	9116006663	
11	Athrav	219301466	Btech cse	Hosteler	9833094011	
12	Shashank Goyal	211002043	Bio science	Hosteler	9024935154	
13	Naman Agrawal	219303093	B.Tech CCE	Hosteler	7013464852	
14	Riddhi Daga	211201064	BJMC	Hosteler	9331214622	
15	Vivek Anand	210901312	Business Administration	Hosteler	8610310054	
16	Yashi Shree	229301030	Btech	Hosteler	9599147349	
17	Dr. Subhash Devrath	-	-	Day scholar	9571188767	
18	Mrs. Suman Devrath	-	-	Day scholar	9571188767	
19	Tejashwini joshi	210901112	BBA marketing	Hosteler	6309335977	
20	Harshita mandhra	220501018	Architecture	Hosteler	9610814620	
21	Vaishnavi shukla	210501022	Barch	Hosteler	7607694292	
22	Anaya	221151002	1 year phd	Hosteler	7889559667	
23	Abhik	220502004	M.arch	Day scholar	7873726178	
24	Ankita Shrivastava	220501012	B. arch	Day scholar	8839638509	
25	PRACHITA BHIWAPURKAR	200501001	B.ARCH	Hosteler	9898711500	
26	Aarshia Chauhan	229302370	BTech IT	Hosteler	9710000136	
27	Vaidehi Agarwal	229302245	Btech IT	Hosteler	9999367467	
28	Arpita garg	229303156	Btech with cce	Hosteler	7983182007	
29	Shriya	220501014	B.arch	Hosteler	9599571767	
30	Kasvi Soni	229311033	Btech cse with iot	Hosteler	9650848355	
31	MOULESH MR	220501005	B arch	Hosteler	9087023888	
32	Rudr Sikaria	220801018	BHM	Hosteler	8638136126	
33	Ahaana Verma	221015001	BCA	Hosteler	9315421451	
34	Mehma singh	220801003	Bhm management	Hosteler	7267984000	
35	Yash bhargava	221201025	BAJMC	Hosteler	8458922968	
36	Divyansh	229310407	CSE(AI&ML)	Hosteler	7082947781	
37	Anjali choudhary	211002005	Bsc biotechnology	Hosteler	6367051288	
38	Ikshita Bagla	220501021	B.Arch	Hosteler	9336057274	
39	Sajal panwar	220501002	B.arch	Hosteler	9667899121	
40	Mustansir kanchwala	220903021	B.com honours	Hosteler	8871600661	
41	Rijul Chaudhary	220501003	B.arch	Hosteler	8433130649	
42	Aarya Chandiramani	220501010	B.arch	Day scholar	8852953085	
43	Arghya Bhagwat	220501022	B.Arch	Hosteler	8219847663	
44	Pranjal Puri	220606004	B.des interior design	Hosteler	7727031282	
45	Vedika Gupta	221007014	BSc psychology	Hosteler	9310489974	
46	Shinaya Badgujar	221105022	BA Liberal Arts	Day scholar	8209657590	
47	Ayamullah Khan	229309022	B.Tech	hosteler	8530044774	

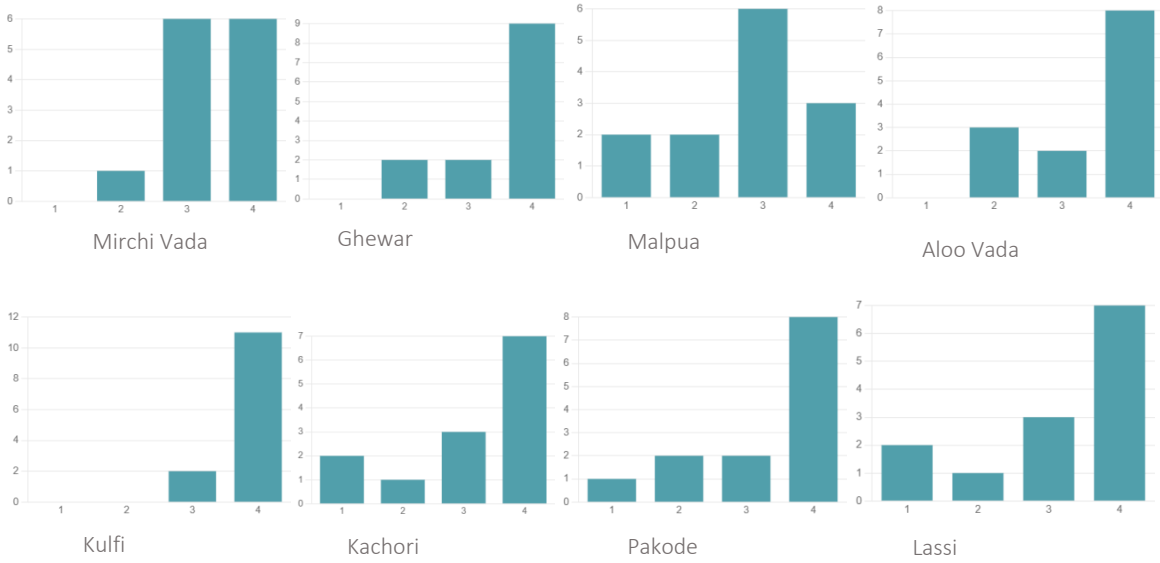
8. Feedback:

Students were amazed by the flavor of cuisines and had a boundless experience while exploring local markets and historical sites through the organized route. They cherished and gave a positive response towards organizing such walks and events in future.

Following is the feedback collected through Google Forms-

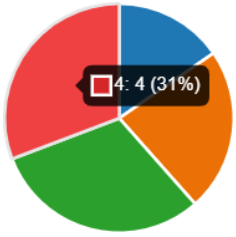
Response to each food items by students-

1-Not Bad; 2-Good; 3-Very Good; 4-Delicious

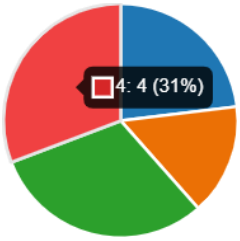


- Route curated for the walk-

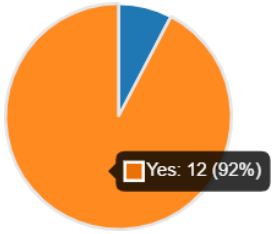
  1-Very Satisfied; 2-Satisfied; 3-Neutral; 4-Unsatisfied



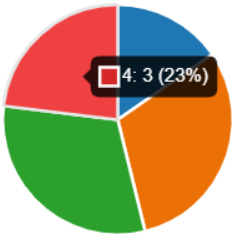
- Walk to be informative-



- Order of the Food Items-



- Overall Experience-



- Feedbacks from Students through forms-

  "Walk was well organized". – Bhavesh Khemka

"Good event... Enjoyed very much". – Rudr Sikaria

"It was a very great experience. Tasting different food items I have never heard of was superb". - Mansi

9. Post event link-

10. Faculty Coordinator:

Signature of Faculty Coordinator
Ar. Ayushi Sharma
Assistant Professor, SA&D
9660311113

Signature of Faculty Coordinator
Ar. Neha Saxena
Associate Professor, SA&D
9950158160

Assistant Director, DSW

DIRECTOR STUDENT WELFARE & PROCTOR
MANIPAL UNIVERSITY, JAIPUR

MANIPAL UNIVERSITY
JAIPUR

MUJ/Q&C/22/F/1.01

MANIPAL UNIVERSITY
JAIPUR

FACULTY OF SCIENCE

SCHOOL OF BASIC SCIENCES

DEPARTMENT OF BIOSCIENCES

INDUSTRIAL WORKSHOP ON THE OPERATIONS AND HANDLING OF BIOREACTOR

Date of Event (11.09.2023-12.09.2023)

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1. **Introduction of the Event-** In this workshop, we get the essential knowledge and practical skills about the bioreactor. A bioreactor is a specialized device used in biotechnology and microbiology to create controlled environments for the growth and cultivation of various biological organisms, primarily microorganisms such as bacteria, yeast, and fungi, as well as cells and tissues. These versatile devices play a pivotal role in various scientific and industrial applications, including pharmaceuticals, biopharmaceuticals, agriculture, environmental remediation, and biofuel production.

2. **Objective of the Event**

   - ***Understanding Bioreactor Principles*:** Fundamental understanding of bioreactor principles, including how they work, their components, and their role in industrial processes.

   - ***Bioreactor setup and monitoring*:** Bioreactor setup and monitoring are crucial aspects of bioprocess management, ensuring the controlled cultivation of microorganisms, cells, or tissues for various applications.

   - ***Safe Handling and Operation*:** Safe handling of bioreactors, emphasizing the importance of following safety protocols to prevent accidents and ensure the well-being of personnel and the environment.

   - ***Process Optimization*:** Optimizing bioprocesses within bioreactors, including parameters like temperature, pH, agitation, and aeration, to maximize productivity and yield.

3. **Beneficiaries of the Event:** Gain in-depth knowledge and practical skills related to bioreactor setup, operation, and monitoring. This knowledge is beneficial in many food industries. with a better understanding of bioreactor monitoring and control, we can maintain consistent product quality, a crucial factor in industries such as biopharmaceuticals where product safety is paramount.

4. **Details of the Guests**

   Mr. Abhishek Thakur is an engineer at PRS BIO

5. **Brief Description of the event:**

   The "Industrial Workshop on the Operations and Handling of Bioreactor" is a specialize ed event designed to provide comprehensive knowledge and practical insights into the setup, operation, and management of bioreactors in industrial settings. This workshop

aims to cater to professionals, scientists, researchers, engineers, and individuals across various industries and sectors where bioreactor technology plays a crucial role.

Key elements of this workshop typically include:

In-Depth Learning: The event offers participants a deep dive into the principles, components, and operational aspects of bioreactors. Attendees will gain a thorough understanding of how bioreactors work and their importance in various industries.

Safety and Regulatory Compliance: Safety is a paramount concern when working with bioreactors. The workshop provides guidance on safe handling practices and emphasizes compliance with industry regulations and standards.

Hands-On Experience: The workshop focuses on the opportunity for practical, hands-on experience with bioreactor equipment, allowing them to apply their knowledge in a real-world setting.

Process Optimization: The workshop focuses on strategies and techniques for optimizing bioreactor processes, including monitoring and controlling critical parameters like temperature, pH, agitation, and aeration.

Quality Assurance: Quality control and assurance are essential in industries like pharmaceuticals and biopharmaceuticals. The workshop covers methods for ensuring product quality and consistency.

Industry Insights: The workshop features presentations, case studies, and discussions on current industry trends, innovations, and best practices related to bioreactor technology.

Practical Applications: The knowledge gained from the workshop can be directly applied to various sectors, including pharmaceuticals, biotechnology, environmental science, agriculture, and food production.

Career Development: Individuals attending the workshop can enhance their skills and knowledge, potentially opening up new career opportunities and advancement prospects in their respective fields.

Overall, the "Industrial Workshop on the Operations and Handling of Bioreactor" is a valuable educational and networking event that equips participants with the expertise and confidence to operate bioreactor systems effectively, driving advancements in biotechnology, pharmaceuticals, environmental science, and related fields.

6. Photographs

Dr. Sandeep Srivastava and Dr. Rakesh Kumar Sharma introduced our guest and briefed the workshop.

Our Guest Mr. Abhishek Rathore demonstrated the bioreactor controls

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7.Brochure of the event:

8. Schedule of the event

DATE	TIMINGS	TOPIC
11/09/2023	10:00 AM	Inauguration
	10:30 AM	High Tea
	10:45 AM	Basics of Bioreactor
	11:30 AM	Reactor components
	1:00 PM	Lunch Break
	2:30 PM	Reactor setup
12/09/2023	9:30 AM	Bioreactor and data acquisition
	1:30 PM	Lunch Break
	2:30 PM	Q&A Session

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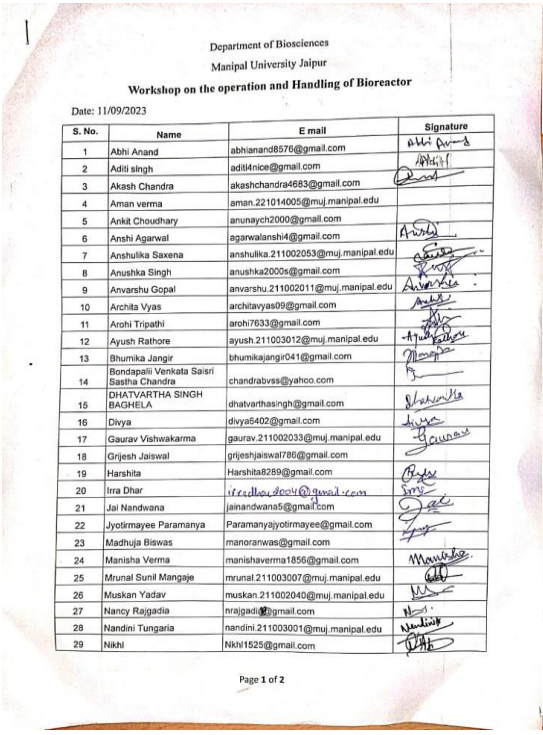
Attendance of the Event

Total attendee-71



Department of Biosciences
Manipal University Jaipur

Workshop on the operation and Handling of Bioreactor

Date: 11/09/2023

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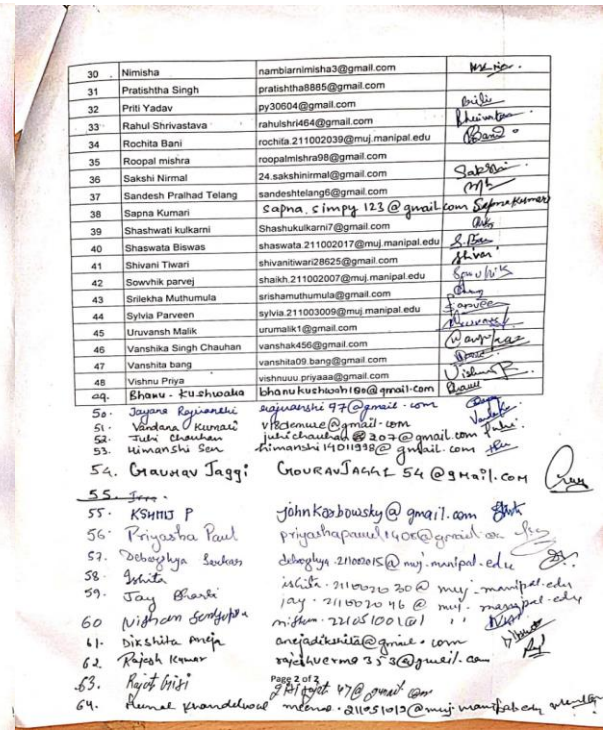

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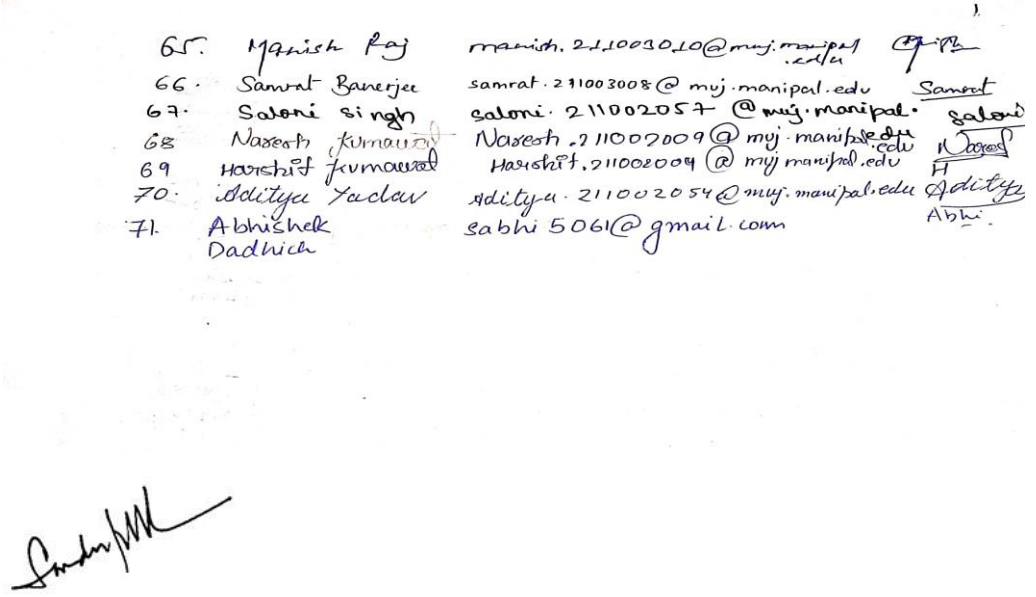

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Seal and Signature of Head with date

MANIPAL UNIVERSITY JAIPUR

FACULTY OF MANAGEMENT & COMMERCE

SCHOOL OF HOSPITALITY AND TOURISM MANAGEMENT

&

Directorate of International Collaboration, MUJ

in collaboration with

IAESTE LC MUJ

along with

JoJo Internationals

has organized a

CULTURAL CULINARY WORKSHOP

(SDG: Zero Hunger & Sustainable Consumption)

11/08/2023

(9:00 – 14:00)

AMIT DATTA

Digitally signed by AMIT DATTA
Date: 2024.01.11 11:36:03 +05'30'

Content of Report

1. Introduction

Goal 12 is about ensuring sustainable consumption and production patterns, which is key to sustain the livelihoods of current and future generations. Our planet is running out of resources, but populations are continuing to grow. The workshop also aims to generate awareness of it. Goal 2 is about Zero Hunger, which is a key to sustain humanity and provide nutritious food for all human being.

The International Association for the Exchange of Students for Technical Experience, Association, commonly known as IAESTE, is an association of national committees representing academic, industrial and student interests. The main aim is to help the members realise their dreams and to facilitate the exchange of ideas both technical as well as, cultural, by connecting students from various cultures and countries.

2. Objective of the Event

- To promote and strengthen international collaborations at MUJ
- To provide a learning opportunity for the SHTM students
- Spread SDG Awareness related to Responsible Consumption & Production and Zero Hunger

3. Brief Description of the event

On 11th August 2023, a Cultural Culinary Workshop was organized in association with IAESTE-MUJ and JoJo International. The DoIC, MUJ supported the event. IAESTE interns from 22 different countries and 25 BHM students participated in preparing their local delicacies. The workshop was organized at SHTM lab. A session on sustainable development

goals 12 and 2, i.e on Sustainable Consumption & Production and Zero Hunger was conducted by the resource person and SHTM faculty members. Further the career scope of culinary professional was shared with the students. Thereafter the participants prepared different dishes. The food was presented and the leadership team of MUJ tasted and applauded the efforts of the participants. Later certificate was awarded to all the participants.

4. Photographs of the event

Glimpses of the event

Certificate distribution to the foreign IAESTE interns

3. SDG Presentation by the resource person foreign interns

5. Brochure

MANIPAL UNIVERSITY JAIPUR

IAESTE LC MUJ

JOIN INTERNATIONAL

SCHOOL OF HOSPITALITY AND TOURISM MANAGEMENT

&

Directorate of International Collaboration, MUJ

Has organized a

Cross -Cultural Culinary Workshop

On 11th August 2023 (SHTM Lab)

For Registration Contact IAESTE coordinators:
Yahya Aseerullah (9573642592) or Aditya Patil (9421524060)

3 Brochure

MANIPAL UNIVERSITY JAIPUR

6. Schedule of the event

Resource Person: Mr Ankit Adhikari, Recruitment Supervisor, JoJo International. Email: cv8@jojointernational.co.in. www.jojo-international.com.au (+61470234428)

Date	Time	Duration	Venue
11th August, 2023	9:00 am – 14:00 pm	05 hours	#325, 1AB HM Lab
Introduction, Culinary Session on Responsible Consumption & Production and Zero Hunger, Food Presentation, Certificate Distribution, Lunch, Vote of thanks.			

7. Attendance of the Event Total attendee – 47 (22 Foreign + 25 Indian [MUJ])

Sr	Participant's Name	Country	University
1	Aaron John Goff	United Kingdom	University of Edinburgh
2	Marlene Elisabeth Metz	Germany	Heidelberg University
3	Benedikt Lohnes	Germany	Technical University of Darmstadt
4	Nina Lauks	Poland	Uniwersytet Medyczny w Lodzi
5	Yaba Rosette Tanoé	Germany	Friedrich-Alexander-Universitat Erlangen-Nurnberg
6	Suwapat Thongyoun	Thailand	Chulalongkorn University, Bangkok
7	Blanca Prior Palomero	Spain	Universidad Politecnica de Madrid
8	Valentín Gregorio Galindo Benéitez	Spain	Universidad Politecnica de Madrid
9	Friedrich Albrecht Dang	Germany	Technische Universitat Munchen
10	Mustafa Aidini Abala	Turkey	Erciyes University
11	Tristan Robert A. Toye	Belgium	Katholieke Universiteit Leuven
12	Laura Maria Estrada D'Amado	Sweden	Chalmers University of Technology
13	Pablo Rodriguez Sanchez	Spain	University of Málaga,
14	Arshia Vali Pour	Iran	Iran University of Science and Technology
15	Oscar Monje Lola	Spain	Universidad Politecnica de Madrid
16	Mohamed Haroun Boutaieb	Tunisia	National School of Architecture and Urbanism
17	Khadijeh Ahmadi Zamani	Iran	K.N. Toosi University of Technology
18	Amine Zribi	Hungary	Eotvos Lorand University
19	Eya YAHYAOUI	Tunisia	National Engineering School of Tunis (ENIT)
20	Muhammed Yasir Yılmaz	Turkey	Istanbul Technical University
21	Eren Asci	Turkey	Kocaeli University
22	Daniel Manuel Allan Werner-Meier	Germany	Technical University of Cologne
23	VANSHIKA	India	Manipal University Jaipur
24	MEHMA SINGH	India	Manipal University Jaipur
25	HARSH ADITYA SINGH RATHORE	India	Manipal University Jaipur
26	SHIVAM JAISWAL	India	Manipal University Jaipur
27	ABHIJEET ARORA	India	Manipal University Jaipur
28	AJAY AHIR	India	Manipal University Jaipur
29	SARTHAK GAUTAM	India	Manipal University Jaipur
30	RITU RAJPUROHIT	India	Manipal University Jaipur
31	ALAM HUSSAIN	India	Manipal University Jaipur
32	GARIMA PANDEY	India	Manipal University Jaipur
33	RUDR SIKARIA	India	Manipal University Jaipur
34	VAIBHAV ENDORIA	India	Manipal University Jaipur

35	DHANUSHWEE L	India	Manipal University Jaipur
36	DIVESH NIMAWAT	India	Manipal University Jaipur
37	PREKSHA MAHESHWARI	India	Manipal University Jaipur
38	ARUSHI RATHORE	India	Manipal University Jaipur
39	PAWAN	India	Manipal University Jaipur
40	HIMANSHU SAINI	India	Manipal University Jaipur
41	ANSHUMAN CHETIA	India	Manipal University Jaipur
42	RUDRARAJ SINGH SISODIA	India	Manipal University Jaipur
43	RANJEET SINGH CHUNDAWAT	India	Manipal University Jaipur
44	ANKIT MANKANI	India	Manipal University Jaipur
45	PRAKASH MANKANI	India	Manipal University Jaipur
46	RITWIK GUPTA	India	Manipal University Jaipur
47	KULDEEP SINGH	India	Manipal University Jaipur

8. Feedback of the Event

The session was interesting and will benefit the student's learnings about the different culinary products and about SDG goals of responsible Production and Consumption and Zero Hunger. Similar views were also expressed by the delegates, IAESTE members, and SHTM students after the session was completed.

9. Letter of Correspondence and Certificates

From: Team Incoming IAESTE LC MUJ <head.incoming@iaestemuj.in>
Sent: Monday, August 7, 2023 5:38:53 PM
To: Dr. Amit Datta [MU - Jaipur] <amit.datta@jaipur.manipal.edu>
Cc: President <president@iaestemuj.in>; Dr. Arun Kumar Poonia [MU - Jaipur] <arunkumar.poonia@jaipur.manipal.edu>
Subject: 4th Edition of International Cross-Cultural Culinary Workshop

Dear Sir,

Please find the details of the International Cross-Cultural Culinary Workshop below:

Date: 11 August 2023
Time: 10:00 AM-2:00 PM
Total guests: 30 (including leadership, faculty, foreign interns and team members)

Certificates will be issued to 25 Hotel Management students and 15 foreign interns as discussed.

Thank you.
--
Warm Regards,

IAESTE LC MUJ

Team Incoming
IAESTE India, LC
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From: cv8@jojointernational.co.in <cv8@jojointernational.co.in>
Sent: Tuesday, August 4, 2023 2:15 PM
To: Dr. Amit Datta [MU - Jaipur] <amit.datta@jaipur.manipal.edu> Dr. Aravind Kumar Rai [MU - Jaipur] <aravindkumar.rai@jaipur.manipal.edu>
Subject: FW: Proposal for Meeting

Respected Sir,

Greetings from JOJO International!!

Hope this email finds you in good health.

Noted and also discuss the benefits and advantages associated with the programs.

Feel free to keep in touch with us about any of your queries.

***NOT HAPPY** with Our Services, **then Write** your **CONCERN at** care@jojointernational.org*

Best Regards,

Ankish Adhikari,
Recruitment Supervisor.
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Think Green Before You Print This Page!!!

From: Dr. Aravind Kumar Rai [MU - Jaipur] <aravindkumar.rai@jaipur.manipal.edu>
Sent: Wednesday, July 28, 2023 5:21 PM
To: cv8@jojointernational.co.in

CERTIFICATES :

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RESEARCH ARTICLE

WILEY

Efficacy evaluation of newly isolated zinc solubilizing bacteria for their potential effect on maize (*Zea mays* L.) under zinc deficient soil conditions

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Abstract

Zinc solubilizing bacteria (ZSB) induces the conversion of fixed and unavailable soil zinc to readily available zinc contributes plant zinc nutrition and fortification. The present research intended to determine the screening of plant growth-promoting (PGP) traits of potent ZSB, biochemical and molecular characterization of ZSB, and assessment of potent ZSB for crop yield at the field level. Therefore, in the present study, molecular and functional characterization of native ZSB isolates was done to examine their response to plant growth performance and yield, mobilization of zinc, and acquisition by maize plants. Zinc solubilizing bacterial isolates namely, ZSB1, and ZSB 17 were solubilized insoluble zinc namely, $ZnCO_3$, ZnO, $Zn_3(PO_4)_2$ and significantly induced growth performance of maize crop at field conditions. A biochemical study revealed that both ZSB isolates were positive for catalase and urease production. Isolates ZSB1 & ZSB17 showed different PGP attributes like production of Indole-3-acetic acid (IAA), siderophore, NH_3, and HCN. Both isolates were solubilized phosphate, potassium, and silica and showed 1-aminocyclopropane-1-carboxylate (ACC)-deaminase activity. 16S rRNA amplification and sequence study of ZSB1 and ZSB17 revealed that both the isolates were *Cupriavidus* sp. and *Pantoea agglomerans*, respectively, and novel. The results elucidated from pot studies demonstrated that both ZSB1 & ZSB17 were the more suitable isolates than other ZSB isolates, and these isolates were further tested for field studies. *Cupriavidus* sp. and *Pantoea agglomerans* strains increased Zn-translocation toward grains and yield of Maize (cv: P3441) by 19.01% and 17.64%, respectively. We conclude that the novel indigenous ZSB strains substantially heightened zinc mobilization, the yield of maize crop, restore soil health, and can be suitable for biofortification and biofertilizers technology.

KEYWORDS
16S rDNA sequencing, field experiment, PGP attributes, zinc solubilizing bacteria, zinc translocation index

1 | INTRODUCTION

The availability of plant necessary elements has a direct impact on soil fertility and agricultural crop productivity. The availability of plant essential elements may change as a result of the buildup of higher concentrations of metals and metalloids in contaminated soil (Alengebawy et al., 2021). A mediated metabolic pathway requires minimal metalloids and heavy metals at appropriate concentrations

for root microbiota, soil fertility, and plant growth (Barra & Terenzi, 2021; Upadhyay et al., 2022). Few metalloids and heavy metals, on the other hand, are even at low concentrations hazardous to plant development and soil fertility (Chibuike & Obiora, 2014). Man-made activities such as mining, developing industrial zones, chemicals and pesticides, waste disposal, and so forth are increasing the prevalence of these contaminants (Alengebawy et al., 2021; Upadhyay & Edrisi, 2021).

Essential elements such as Zn (zinc), Cu (copper), Fe (iron), Mg (magnesium), and so forth are necessary to plant growth at an optimum concentration (White & Brown, 2010). Plant growth and soil fertility are also reduced by (i) a higher concentration of essential elements, and (ii) incompatible form of essential elements in the soil (Baldantoni et al., 2019), hence optimum concentration of essential micronutrients is required for soil productivity. Microbes can mobilize or solubilize trapped essential elements in contaminated soil by releasing extra-cellular enzymes; these enzymes may be facilitated by redox reactions (Garcia-Arellano et al., 2004).

Plant growth promoting rhizobacteria (PGPR) plays remarkable and promising role in phyto-stimulation by releasing plant hormones like Indole-3-acetic acid (IAA), Gibberellins and so forth (Upadhyay & Chauhan, 2022), and other solubilized trapped essential elements of soil and increasing essential element uptake in plants (Singh et al., 2022; Upadhyay et al., 2009). These procedures are known as PGPR direct mechanisms (Mahmud et al., 2021; Singh et al., 2022). The production of exo-polysaccharides (Upadhyay et al., 2011), antibiotics, antioxidants (Upadhyay & Singh, 2015), biocontrol action to reduce phytopathogens, and so forth are indirect mechanisms of PGPRs (Mahmud et al., 2021). Mobilization and solubilization of trapped essential elements by rhizobacteria can be effective sustainable approaches to improving plant growth performance and enhancing soil fertility in zinc-contaminated soil (Bhojiya et al., 2022).

The ZSB (zinc solubilizing bacteria) are renowned for their effectiveness in the solubilization of zinc when combined with plant root exudates, which function as a chemo-attractant and improve the availability of native rhizobacteria promotes plant growth (Upadhyay et al., 2022). ZSB thus facilitate native zinc for plant assimilation, leading to plant growth promotion (Shakeel et al., 2015). Previously, studies on the utilization of ZSB to enhance the Zn acquisition in crops such as wheat, mung-bean etc. and correcting Zn deficiency in soil by increasing over 50% available Zn levels in the harvest soil samples has been reported (Dinesh et al., 2018; Mumtaz et al., 2017; Sirohi et al., 2015). In more than 300 enzymes, zinc and zinc ion plays a vital biological role by maintaining protein structure & stability and is found in many metalloenzymes as essential cofactor (Sarathambal et al., 2010).

Zinc deficiency leads to biomass and fertility reduction directly reduces crop plant yield, chlorosis in leaves which negatively impact photosynthesis, increased iron accumulation causing cellular toxicity, and increased oxidative stress with reduced Cu/Zn SOD activities (Thiébaut & Hanikenne, 2022). Zinc deficiency in maize is very likely to result in stunting, acute chlorosis, reduced pollen viability, and male sterility (Brown, 2008). Due to the selective cultivation of high-yield maize varieties with synthetic fertilizers to boost cropping and quality over the past few decades, zinc deficiency has ravaged into the soil-crop environment, making maize the most susceptible cereal crop to Zn deficiency (Fageria et al., 2002).

Fifty percent of global and Indian soils are zinc deficient which is projected to increase to an estimated 63% by 2025 leading to reductions not only in crop yield but also in food quality (Hussain et al., 2022; Shukla et al., 2021). In India, 51.2% soils from the states Andhra Pradesh, Assam, Bihar, Chhattisgarh, Goa, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Odisha, Rajasthan, Telangana, and Uttar Pradesh were deficient in available Zn (Shukla et al., 2021). Zn solubilization and mobilization by soil microbes has sustainable perspectives in comparison to chemical fertilizers. Therefore, the intent of current investigation was focused on (i) isolation and screening of potent ZSB and its plant growth-promoting (PGP) attributes, (ii) 16SrRNA characterization of potent screened bacterial isolates, (iii) Influence of potent isolates on plant growth and soil health in zinc infested soil at field level.

2 | MATERIALS AND METHODS

2.1 | Physico-chemical properties of rhizospheric soil samples

The rhizospheric soils of chickpea plant were obtained from the Dungarpur (23.85° N; 73.68° E) and Pratapgarh (23.56° N;73.74° E) districts of Rajasthan (Figure 1), both the sites were adjacent to ZAWAR mines (Latit-24.3540034; Long-73.733064). Physico-chemical properties such as EC (Electrical conductivity), OC (Organic Carbon), Av. N (available nitrogen), Av. P (available phosphorus), Av. K (available potassium), and diethylenetriaminepentaacetic acid (DTPA) extracted zinc were analyzed as per standard procedures (Jain, Kour, et al., 2020; Vance et al., 1987).

2.2 | Isolation of ZSB and screening of its zinc solubilizing potential

The ZSB isolation was done with serial dilution plate method on specific media namely, Mineral salt media (Saravanan et al., 2007) and Bunt & Rovira medium (Bunt & Rovira, 1955) supplemented with different insoluble zinc source such as ZnO, $ZnCO_3$, and $Zn_3(PO_4)_2$ to produce a clear halo zone after 48 h incubation at 28°C ± 2°C were purified and considered as ZSB. To evaluate zinc solubilization efficiency of the isolates, the halo zone forming bacterial isolates were put on Bunt and Rovira agar and MSM media plates with a 0.1% insoluble zinc-source and at 28°C ± 2°C plates were incubated for 48 h. Zn solubilization efficiency was calculated as given equation.

$$\text{Solubilization efficiency} = \frac{\text{Zone diameter}}{\text{Diameter of colony growth}} \times 100$$

Further, for quantitative estimation (broth assay) of zinc solubilizing potential of ZSB strains were determined by following Gandhi

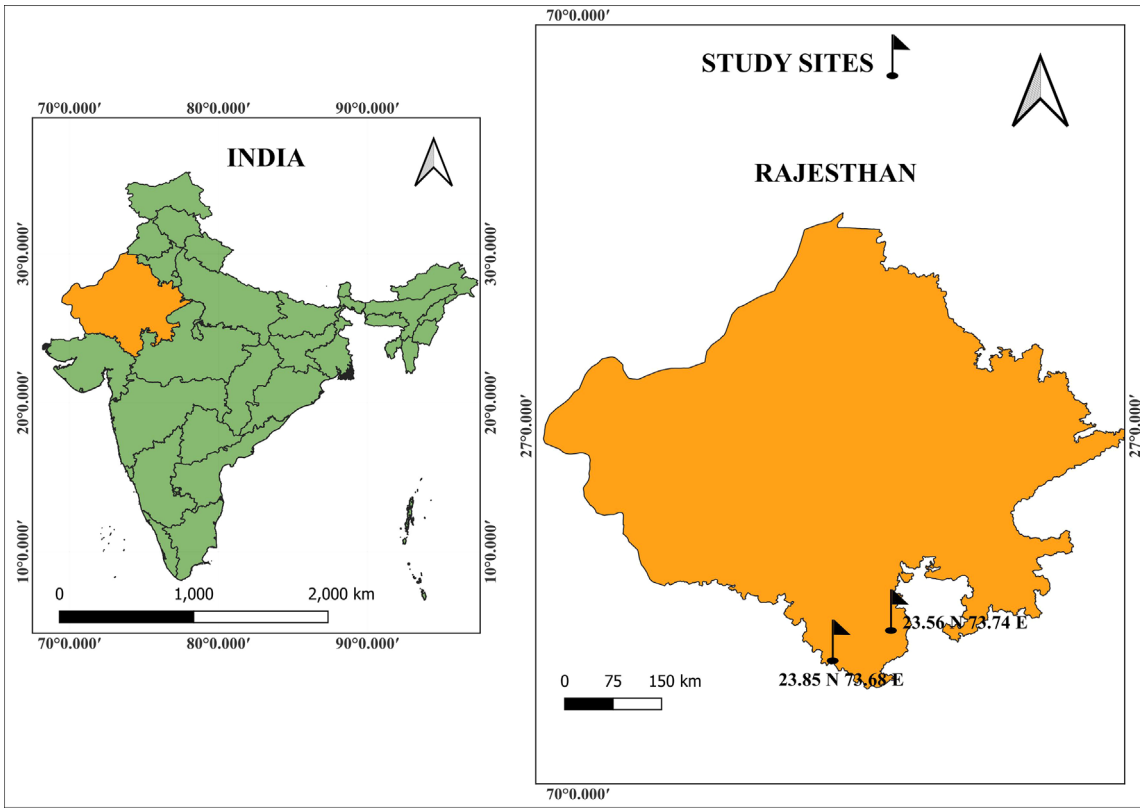

FIGURE 1 Map of the state of Rajasthan showing the geographic locations of collection of soil samples for the isolation of zinc solubilizing bacteria. [Colour figure can be viewed at wileyonlinelibrary.com]

et al. (2014). Briefly, the available zinc concentration was measured using atomic absorption spectrophotometer (AAS 4141 model, Electronics Corp. of India Ltd., India) in the culture filtrate of ZSB grown in MSM broth containing different insoluble zinc source (0.1%) at 4th, 8th, and 16th day of incubation (Gandhi et al., 2014). The pH shift of culture filtrate and uninoculated medium were also analyzed using pH meter.

2.3 | Morphological, biochemical, and molecular identification of potent ZSB

Morphological characteristics namely, form, elevation, margin, cell form, colony color, appearance colony morphology, growth, Gram staining (Gram, 1884) and basic biochemical test namely, Catalase test, Urease test, and Gelatin Liquification test were studied using the standard procedure (Blazevic & Ederer, 1975). Molecular identification of the screened ZSB isolates was carried out through 16S rRNA PCR amplification by using universal primers according to Weisburg et al. (1991) and Jain, Sanadhya, et al. (2020) and sequenced. The 16S rDNA sequences of ZSB isolates were subjected to a BLAST analysis (Altschul et al., 1990) in order to retrieve closely related sequences of type strains and further aligned using online tool CLUSTAL-W (Thompson et al., 1994). The MEGA 6.06 software was employed to construct phylogenetic tree (Tamura et al., 2013).

2.4 | HPLC and GCMS analysis for gluconic acid

The production of gluconic acid by ZSB isolates were tested by injecting the 5 days pre incubated culture filtrate in to a RP-HPLC (Agillent) having C18 column and the mobile phase acetonitrile: water (30:70 v/v) with a flow-rate @ 1.0 mL/min was used with an isocratic flow to detect gluconic acid at 210 nm through UV/Vis-detector (Jain, Kour, et al., 2020). The culture filtrates were further evaluated for the presence of various organic acids and other moieties using GCMC (GCMSQP2020, Shimadzu). Briefly, the methanol extracts (500 µL) of lyophilized culture filtrate 100 µL of N-Methyl-N-(trimethylsilyl) trifluoroacetamide and 100 µL of pyridine were added and the reactions were heated (60°C for 30 min gently) in a water bath and left 12 h for stabilization. These processed samples were analyzed through GC–MS (source temperature 200°C, ionizing voltage 70 eV) and operated with scan mode (50–700 m/z) with temperature ranged 70–260°C and data was compared with NIST library.

2.5 | Physiological and PGP attributes of potent ZSB

Physiological attributes of potent ZSB isolates such as tolerance of pH (Graham, 1992), tolerance of salinity (Upadhyay et al., 2009) tolerance of temperature (Graham, 1992), tolerance of drought (Abolhasani

et al., 2010), antibiotic resistance (Li & Ramakrishna, 2011) was performed by using standard protocols. Zinc solubilizing bacterial isolates were examined for their multiple PGP traits such as production of IAA, siderophore-production, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, phosphate-solubilization, potassium and silica solubilization, HCN, ammonia and exopolysaccharides production with standard published methodologies (Jain, Kour, et al., 2020; Naureen et al., 2015; Siddiqui et al., 2021; Upadhyay et al., 2011; Yadav et al., 2022). Hydrolytic enzymes (αamylase, cellulase, pectinase, and protease) was measured by the method of Cappuccino and Sherman (1992) & lipase (Ertugrul et al., 2007), chitinase activity (Kumar et al., 2012), and glucanase activity (Fawzy and Monaim, 2016) were screened by using standard protocols.

2.6 | Bio efficacy evaluation of potent ZSB

2.6.1 | Pot experiment

Bio efficacy and plant growth promotion ability of selected ZSB1 and ZSB17 strains as liquid microbial inoculants was evaluated under pot culture in triplicate following complete randomized design according to our previously published research (Jain et al., 2021). The maize seeds (5–10) were treated with ZSB liquid inoculants (>8.5–$\times 10^8$ cfu mL^{-1}) and placed in 4.0–5.0 cm deep in each pot. All the pots were given uniform recommended dose of fertilizers (RDF) namely, N (@ 120 kg N: P@ 60 kg P_2O_5 and K @ 40 kg K_2Oha^{-1}; Omara et al., 2016). After 30 days of sowing, plant growth parameters namely, average shoots, root-length, root-number, leaf-number, and leaf chlorophyll content (Ronen & Galun, 1984) were analyzed using standard protocols.

2.6.2 | Field experiment

The field studies were undertaken at Krishi Vigyan Kendra, Dungarpur and Instructional farm, Rajasthan College of Agriculture (RCA), (composite soil analysis reports of both experimental fields were summarized in Supplementary data sheet Table S1.1), where the DTPA extractable zinc content is low (<0.6 PPM) in 2 years of kharif seasons to differentiate the effect of two ZSB isolates on growth and yield of Maize variety P3441. The field experiment was laid out in a RBD (randomized block design) with 15 treatments in three replications including two ZSB isolates and uninoculated control (S_1: 100% RDF, T_1: ZSB1 ONLY, T_2: ZSB1+ 100% RDF, T_3: ZSB1 + 75% RDF, T_4: ZSB1 + 50% RDF, T_5: ZSB1 + 100% RDF + $ZnSO_4$, T_6: ZSB1 + 75% RDF + $ZnSO_4$, T_7: ZSB1 + 50% RDF + $ZnSO_4$, T_8: ZSB17 ONLY, T_9: ZSB17 + 100% RDF, T_{10}: ZSB17 + 75% RDF, T_{11}: ZSB17 + 50% RDF, T_{12}: ZSB17 + 100% RDF + $ZnSO_4$, T_{13}: ZSB17 + 75% RDF + $ZnSO_4$, T_{14}: ZSB17 + 50% RDF + $ZnSO_4$) as similar approach was adopted by earlier reported work of Upadhyay et al. (2019). The sowing was done by manual dibbling the seeds at a distance of 60 cm × 40 cm row to plant (Fahad et al., 2016).

ZSB liquid biofertilizer @ 5 mL kg^{-1}treated to seed before sowing. To enhance the health of cropping over the crop season, all recommended agronomical practices namely, sowing, weeding, manuring, harvesting, and so forth were taken. Ten plants were randomly selected from every plot at physiological maturity of the crop (106–110 days from sowing), the parameters of yield and harvest including cob length (cm); number of grains per row; number of rows per cob; weight of cobs per plot; weight of grain (g); thousand grain weight (g); biological yield per plot (g); harvest index (%) were evaluated manually (Supplementary data sheet: experimental details) (Gheith et al., 2022). Data analysis was accomplished by using the analysis of variance determining levels of significance.

2.7 | Analysis of Zn-content and Zn-translocation index (ZTI)

The powdered sample (shoot and grain) from all 15 treatments were digested using a triacid mixture (HNO_3: H_2SO_4: $HClO_3$ in the ratio of 9:2:1) and the Zn-content were measured using AAS to quantify the Zn translocation index (ZTI) (Rengel & Graham, 1996).

$$\mathrm{ZTI} = \frac{\text{Zn concentration in grains}}{\text{Zn concentration in shoot}} \times 100$$

3 | RESULTS

In the present study, the physico-chemical characteristics of Dungarpur and Pratapgarh soil samples are described in Table S1.2. The soil samples textured with clay loam and sandy loam, while the soil pH ranged from acidic to neutral. The rhizospheric soils contains moderate to high range of ECe, OC, Av. N, Av. P, and Av. K. The DTPA extractable concentrations of Zn-soil (available Zn) were observed as 0.572 and 0.686 ppm.

3.1 | Isolation and assay (qualitative and quantitative) for zinc solubilization by ZSB

Microorganisms have varied solubilization response with different insoluble form of zinc hence, in the present study, ZSB isolates ZSB1 and ZSB17 were selected based on their capabilities in solubilizing multiple forms of insoluble zinc namely, ZnO, $ZnPO_4$, and $ZnCO_3$ in plate assay. Qualitative screening of zinc solubilization was carried out in MSM media and R&B media plates supplemented with different insoluble Zn compounds (Table 1). Zn solubilization zone with ZSB1 was observed in MSM media plates was 3.78 mm, 5.46 mm and 4.10 mm with $ZnCO_3$, ZnO, and $Zn_3(PO_4)_2$, respectively, and by ZSB17 was 3.09 mm, 3.79 mm, and 6.56 mm with $ZnCO_3$, ZnO, and Zn3 $(PO_4)_2$, respectively whereas in R&B media maximum zone of solubilization was observed with ZSB1 was 3.78 mm, 5.43 mm, and 4.10 mm with $ZnCO_3$, ZnO, and $Zn_3(PO_4)_2$, respectively, and by ZSB17 (3.09 mm, 2.85 mm, and 6.56 mm with $ZnCO_3$, ZnO, and $Zn_3(PO_4)_2$, respectively). Higher solubilization of Zn was observed in plates containing MSM media.

TABLE 1 Qualitative and quantitative assay for Zinc solubilization by ZSB strains on different insoluble Zn compounds.

	Qualitative assay for zinc solubilization by measuring solubilizing index (SI)					
	SI ON R&B (ZNO)	SI ON R&B (ZNC)	SI ON R&B (ZNP)	SI ON MSM (ZNO)	SI ON MSM (ZNC)	SI ON MSM (ZNP)
ZSB-1	5.43 ± 0.05	3.78 ± 0.02	4.1 ± 0.02	5.46 ± 0.05	3.78 ± 0.02	4.10 ± 0.02
ZSB-17	2.85 ± 0.04	3.09 ± 0.08	6.56 ± 0.01	3.79 ± 0.02	3.09 ± 0.08	6.56 ± 0.01

	Qualitative assay (broth assay) by measuring soluble Zinc (µg/mL) using AAS			
	4th day (µg/mL)	8th day (µg/mL)	16th day (µg/mL)	pH
ZSB-1	5.1800 ± 0.0436	14.5767 ± 0.0416	17.3033 ± 0.0603	30.2
ZSB-17	6.1100 ± 0.0201	14.2500 ± 0.0657	14.6533 ± 0.6240	40.1

Abbreviations: MSM, mineral salt media; R&B, bunt & Rovira medium; ZNO, Zinc oxide; ZNC, Zinc carbonate; ZNP, Zinc phosphate.

Both ZSB strains were further evaluated for quantitative Zn-solubilization at different time intervals in MSM broth (broth assay). The results raveled that the amount of Zn solubilized from insoluble zinc-oxide, zinc-carbonate, and zinc-phosphate by both the ZSB isolates, and Zn solubilization rate was proportional with incubation time (Table 1). Maximum available Zn registered by ZSB1 was 5.18 µg mL^{-1} on the fourth day, which peaked to 14.57 µg mL^{-1} during the eighth day, followed by 17.30 µg mL^{-1} during the 16th day whereas zinc solubilization by ZSB17 was 6.11 µg mL^{-1} on the 4th day, which peaked to 14.25 µg mL^{-1} during the eighth day, followed by 14.65 µg mL^{-1} during the 16th day. Zn solubilization and reduction in pH of the culture medium showed positive correlation for both the ZSB isolates.

3.2 | Morphological, biochemical, and molecular characterization of ZSB isolates

The shape of ZSB1 and ZSB17 isolate was rod and cocci respectively, while both were gram negative. Colony characteristics as colony color, form, elevation, margin and appearance were also noted along with key biochemical tests and described in Supplementary data sheet Table S2. Biochemical analysis revealed that both ZSB isolates were negative for gelatin liquification test, while both were positive for catalase and urease production. The 16S rRNA gene sequence of isolate ZSB1 showed 95.49% homology with 16S rRNA sequence of *Cupriavidus campinensis* strain BT HNGU56 (Accession number KY010351) already submitted to GenBank data repository of the NCBI. The sequence of 16S rRNA gene of isolate ZSB17 showed 99.68% homology with 16S rRNA sequence of *Pantoea* sp. strain AS-43 (Accession number OL604306) already submitted to GenBank data repository of the NCBI [ZSB1: *Cupriavidus* sp. (Accession number: KY244144); ZSB17: *Pantoea agglomerans* strain ZSB17 (Accession number: MK773870)]. The phylogenetic position of the species is shown in Figure 2.

3.3 | Gluconic acid production by potent ZSB isolates

The reduction of pH from in broth assays was validated by measuring gluconic acid from the chosen ZSB isolates using HPLC (Supplementary data sheet Figure S1). Both the ZSB isolates showed the secretion of gluconic acid on comparison with the standard gluconic acid and ZSB 1 and ZSB17 produced 286.14 and 102.74 mg/mL gluconic acid respectively after 5 days of incubation in Zn-supplemented MSM media. Further the culture filtrates were subjected to GCMS analysis which also revealed the secretion of different organic acids (Supplementary data sheet Figures S2 and S3).

3.4 | Physiological and PGP attributes of potent ZSB

The isolates ZSB1 and ZSB17 was screened primarily for physiological attributes that includes pH tolerance, salinity tolerance, temperature tolerance, drought tolerance, antibiotic sensitivity (Supplementary data sheet Table S3). Strain ZSB1 was able to tolerate at 1% salt concentration while ZSB17 strain were able to tolerate 2% salt concentration. Both isolates were exposed to temperature stress and ZSB1 was able to grow at various temperatures ranging from 25°C to −40°C while strain ZSB17 showed growth at temperature ranging from 20°C to −45°C. Further the drought tolerant capacities of ZSB were assessed using varying concentration of PEG on MSM-broth and ZSB1 were able to grow upto 40% PEG whereas ZSB17 were tolerated upto 10% PEG. The zinc solubilizing isolates resisted the antibiotics peniciline (µg) and ampicillin (µg) and sensitive toward kanamycin (µg), cefixime (µg), and rifampicin (µg).

Multiple PGPR activities of both ZSB isolates (Table 2) revealed that strain ZSB1 and ZSB17 were suitable plant growth promoting candidates. In the presence of L-tryptophan ZSB1 and ZSB17 produced 64.49 µg/mL IAA and 66.81 µg/mL IAA respectively. Phosphate solubilization by strain ZSB1 was 2.63 ± 0.4 and by strain ZSB17 2.97 ± 0.7 mm diameter around the colonies. Both ZSB isolates were also found positive for potassium solubilization. Both ZSB isolates were able to solubilize potash as forming clear zones in Aleksandrov agar media supplemented with mica. Zone of potash solubilization by strain ZSB1 was 2.86 ± 0.3 mm and by ZSB17 was 3.53 ± 0.02. Both isolates were also subjected for silica solubilization test. Silica solubilization by ZSB1 was 3.83 ± 0.17 and by strain ZSB17 2.64 ± 0.04 mm diameter around the colonies. These selected ZSB isolates have evaluated for different enzymes production by conducting enzyme assays. Research findings showed that both ZSB isolates

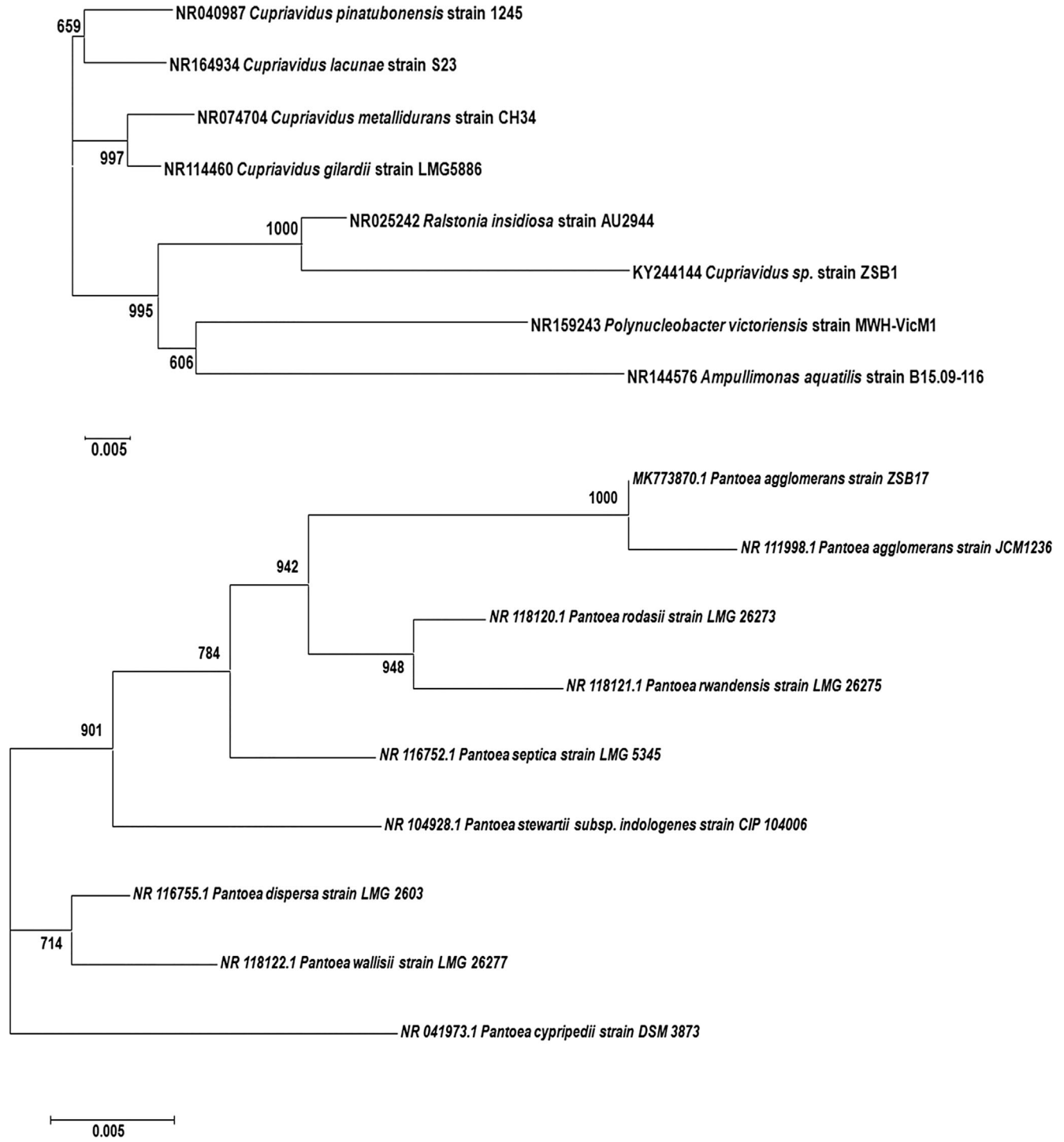

FIGURE 2 Phylogenetic analysis of potent ZSB isolates.

were positive for amylase, lipase, protease, and cellulase production and negative for chitinase and glucanase production with respect to hydrolytic enzymes.

3.5 | Bio efficacy evaluation: Pot and field study

The results from pot experiments reveled that both the ZSB isolates significantly induces maize plant growth-performance. Zinc solubilizing isolates inoculation showed substantial growth in leaf no., leaf-length, shoots-length as compared to uninoculated control and significantly enhanced the root-length, root-number, and leaf chlorophyll content (Supplementary data sheet Table S4). The untreated control showed minimum value in all studied plant growth parameters.

Field experiment was conducted following in-vitro authentication for both selected ZSB isolates ZSB1 and ZSB17 on 13 selected growth and yield related attributes were recorded in Table 3

TABLE 2 PGP and hydrolytic enzyme production traits in ZSB isolates.

Plant growth promoting traits	ZSB1	ZSB17
ACC Deaminase	+	+
Ammonia production	+	+++
Sidero-phore	+	+
HCN	+	+
EPS	−	−
IAA (μg/mL)	640.49	668.17
P solubilization index (cm)	2.63 ± 0.04	2.97 ± 0.07
K solubilization index (cm)	2.86 ± 0.03	3.53 ± 0.02
Si solubilization index (cm)	3.8367 ± 0.17	2.6467 ± 0.04
Lipase activity	+	+++
Amylase activity	+	+
Protease activity	+	+
Cellulase activity	+	+
Chitinase activity	−	−
Glucanase activity	−	−

Note: Value (mean of triplicate) ± standard deviation.

(Supplementary data sheet). In the present research, the preferred maize variety P3441 was used with implementing all favored SAP (standard-agronomic-practices). For field experiment 15 treatments along with control were designed with combination of RDF and $ZnSO_4$. Among all the treatments, the highest biological yield (q/ha) was observed in treatment T_5 (143.82 ± 5.65q/ha) which were combination of 100% RDF, ZSB1 isolates and $ZnSO_4$ followed by treatment T_{12}, T_6, T_{13}, T_2, T_{14}, T_3, T_7, T_9, T_{10}, T_4, T_{11}, T_1, T_8 over the control. The maize plant growth and production have been significantly increased through seed bacterization with ZSB isolates. The difference was significant on yield was recorded in treated than control. Table 3 presents data on the parameters of crop growth and yield trend for maize.

The impact of ZSB isolates on the maize grain Zn content & ZTI are summarized in Table 4. In treatment T_5 (ZSB1+ 100% RDF + $ZnSO_4$) highest ZTI was observed (ZTI = 55.21%) followed by the maize plants treated with treatment T_{12} (ZSB17 + 100% RDF + $ZnSO_4$; ZTI = 53.4%). This clearly illustrates the role of ZSB isolates in translocating Zn toward maize grains. Zinc translocation analysis revealed that zinc acquisition in grain and shoot was significantly enhanced with strain ZSB1 than strain ZSB17 and un-inoculated control.

4 | DISCUSSION

The growth and productivity of crops were significantly impacted by a zinc shortage in the soil ultimately lead to low zinc contents in crops (Hafeez et al., 2013; Hussain et al., 2022). Following previously published studies, the ZSB isolates were obtained from rhizospheric soil in this research (Bhatt & Maheshwari, 2020; Sunithakumari et al., 2016). *Cupriavidus* sp. and *Pantoea agglomerans* were identified as the effective ZSB strains ZSB1 and ZSB17 by 16S rRNA gene sequencing. The biochemical characterization represents the intrinsic biochemical and structural properties of the bacteria to adopt in the specific environment. In medium supplied with zinc phosphate and zinc carbonate, ZSB1 shown higher solubilization efficiency, but ZSB17 demonstrated higher solubilization in medium supplemented with zinc oxide. Ramesh et al. (2014) showed that the findings of the current investigation are supported by the ZSB strains MDSR7 and MDSR14 solubilizing all three zinc compounds (zinc, zinc-phosphate, and zinc-oxide). The current study reports that the higher Zn-solubilization zone was observed in ZnO supplemented medium compared to $ZnCO_3$ amended medium (Goteti et al., 2013; Mishra et al., 2017). In this work, a broth test was used to quantitatively evaluate the solubilization of zinc. As zinc solubilization increased over time, the highest amount of zinc was registered in ZSB17 on day 16 at 14.65 g mL^{-1}. Similar findings with isolated ZSB solubilized insoluble ZnO (40.81 $mL^{-1}$to 62.48 mL^{-1} soluble Zn) were also reported by Mishra et al. (2017). One important mechanism for the solubilization of metals and minerals is the secretion of OA (organic acids) by PGPRs, and gluconic acid is thought to be the main OA involved in the solubilization of insoluble minerals in soil (Sunithakumari et al., 2016). This will be the primary intermediary for solubilization due to the presence of 2-ketogluconic acid as a main product in cultures altered with the solubilization of insoluble zinc source (Gontia-Mishra et al., 2017) and likely as a result of increased acidity (Dinesh et al., 2018).

More or less every organism has a different active mechanism of zinc solubilization, which relies on the type of bacteria present. The ability of the ZSB strains in the current study to withstand stress, including pH, temperature, salt, and drought, is an inherent biochemical characteristic that aids in their survival in challenging rhizosphere conditions (Upadhyay et al., 2019). If a PGPR displays a variety of PGP properties, it might be a good candidate for microbial inoculants (Singh et al., 2022; Upadhyay & Chauhan, 2022). The ZSB1 and ZSB17 strains were positive for multiple PGP traits namely, ACC-deaminase-activity, siderophore-production, HCN-production, and ammonia-production. Rhizobacterial isolates are well established organisms, which may be remarkable assets for plant growth promotion through different mechanisms (Nadeem et al., 2010; Upadhyay et al., 2022; Upadhyay & Singh, 2015). ACC, a precursor for the ethylene stress hormone as the only source of nitrogen plays an important role for plant growth promotion (Mishra et al., 2017). HCN is a secondary metabolite of bacteria that inhibits growth of pathogenic microorganisms (Siddiqui, 2006). Similarly, recently Jain et al. (2020a) demonstrated that zinc tolerant PGPR produce siderophores and induced growth of plants. Ramesh et al. (2014) demonstrated that strong ammonia-producing bacterial isolates can be beneficial as a source of nitrogen for plant growth-performance.

This study, the IAA production capacities of ZSB isolates is consistent with other researchers' findings (Abaid-Ullah et al., 2015; Zhao et al., 2011). Gandhi & Muralidharan (2016) demonstrated that

TABLE 3 Effect of ZSB strains on growth and yield parameters of maize under field experiment.

Treatment	Biological yield (kg)	Cob length (cm)	Weight of cob/plot (kg)	No of cobs/ plot	No of rows/ cob	No of grains/ row	Weight of grain/plot (kg)	Grain yield (q/ha)	Weight of fodder/ plot (kg)	Stover yield (q/ha)	Biological yield (q/ha)	Harvest index (%)	1000 grain wt (g)
S_1	60.40	20.00	40.20	24.00	14.00	40.00	20.59	54.00	30.81	79.31	133.31	40.48	210.65
T_1	40.80	18.00	20.92	22.00	12.00	36.00	20.30	48.00	20.50	51.98	99.98	48.28	245.66
T_2	60.10	22.00	40.10	27.00	16.00	42.00	3.12	65.00	20.98	62.06	127.06	51.26	289.82
T_3	50.90	20.20	30.90	25.00	14.00	36.00	2.83	59.00	30.07	63.90	122.90	48.58	253.00
T_4	50.40	19.00	30.40	24.00	14.00	36.00	20.54	53.00	20.86	59.48	112.48	47.45	254.00
T_5	60.90	23.00	4.58	29.00	18.00	44.00	30.26	68.00	30.64	75.82	143.82	47.23	292.65
T_6	60.40	21.50	40.30	28.00	16.00	40.00	20.98	62.00	30.42	71.31	133.31	46.62	268.00
T_7	50.80	20.00	40.00	26.00	14.00	38.00	20.69	56.00	30.11	64.81	120.81	46.35	278.00
T_8	40.40	17.70	20.46	20.00	12.00	34.00	2.21	46.00	20.19	45.65	91.65	50.19	269.00
T_9	50.80	20.60	30.50	26.00	16.00	40.00	3.02	63.00	20.78	57.81	120.81	52.35	285.00
T_{10}	50.50	19.80	30.20	24.00	14.00	38.00	20.74	57.00	20.76	57.57	114.57	50.03	269.00
T_{11}	50.10	18.40	30.10	23.00	14.00	36.00	20.50	52.00	20.60	54.23	106.23	49.05	288.00
T_{12}	60.60	22.00	40.30	28.00	16.00	41.58	30.07	64.00	30.53	73.48	137.48	47.11	288.51
T_{13}	60.30	21.00	40.10	25.00	16.00	40.00	20.78	58.00	30.52	73.23	131.23	44.50	285.48
T_{14}	60.10	20.50	30.80	23.00	14.00	38.00	20.54	53.00	30.56	74.06	127.06	41.71	255.00
SEm±	00.270	00.937	00.169	10.160	00.638	10.632	00.113	20.362	00.286	50.962	50.602	20.794	90.111
CD at 5%	00.780	20.705	00.488	30.351	10.841	40.712	00.328	60.823	00.827	17.221	16.180	80.069	26.313
CD at 1%	10.051	30.643	00.658	40.513	20.480	60.345	00.441	90.187	10.113	23.188	21.787	10.866	35.431

Note: The data express the pooled value of the triplicate data collected in two sessions.

TABLE 4 Effect of ZSB isolates on Zinc translocation from shoot to grain; Zinc Translocation Index.

Treatment	Zn in grain	Zn in Stover	Zinc translocation index (%)
S_1	25.93	78.60	32.9
T_1	23.20	78.37	29.62
T_2	35.33	68.03	51.9
T_3	33.70	70.79	47.6
T_4	30.30	73.47	41.14
T_5	38.20	68.77	55.21
T_6	34.77	70.00	49.5
T_7	32.97	70.83	46.4
T_8	21.47	79.00	**27**
T_9	34.60	71.23	48.5
T_{10}	31.83	69.80	45.5
T_{11}	27.50	70.33	39.11
T_{12}	35.50	66.33	53.4
T_{13}	31.50	65.73	47.9
T_{14}	29.17	69.03	42.1
SEm±	0.961	1.227	
CD at 5%	2.777	3.543	
CD at 1%	3.739	4.771	

Note: The data express the pooled value of the triplicate data collected in two sessions.

phytohormone IAA (auxin) was produced by AGM3 (an isolate) at 45.61 g mL^{-1}, followed by the AGM9 37.27 g mL^{-1}in IAA broth medium. The capability of PGP isolates to solubilize insoluble P form to a plant available P form significantly improves crop production under P limiting conditions (Majeed et al., 2015).

According to the findings of an experiment performed by Dinesh et al. (2018), *B. megaterium* (Strain CDK25) is capable of soluble and mobilized phosphate, both inorganic and organic. *Bacillus licheniformis* (BHU18) and *Pseudomonas azotoformans* (BHU21), two KSB isolates, demonstrated noticeably higher K-solubilization than the results seen in the current research, according to Saha et al. (2016). According to Naureen et al. (2015), 29 out of a total of 111 bacterial isolates can dissolve mineral silicates. Zhao et al. (2011) reported on the isolation and characterization of ZSB strains with multiple PGP traits and stated that *Bacillus* spp. exhibit numerous plant growth promoting attributes that support plant growth, including Zn and P solubilization, IAA production, oxidase activity, catalase activity, and phytohormone development. The increase in plant growth could be attributed to ZSB isolates' capacity to supply nutrients through nitrogen fixation, phosphate solubilization, siderophores synthesis, and the release of phytohormones (Mumtaz et al., 2017; Jain et al., 2017). Amylase, lipase, protease, and cellulase synthesis were found in zinc solubilizing isolates, and these enzymes indirectly aid plant growth by controlling soil-borne phytopathogens (Jha et al., 2012).

Zinc solubilizing isolates inoculation under pot conditions significantly improved the root length, root no., and leaf chlorophyll content and the results were well supported by Karnwal (2021) reported zinc solubilizing *Pseudomonas* spp. isolated from vermicompost significantly improves plant growth and maximum zinc content in Okra fruit compared to uninoculated control. Application of ZSB substantially improves plant growth by increasing Zn bioavailability in soil to crop plants hence reduce the use of synthetic zinc fertilizers. The field experiment was conducted following in vitro authentication for ZSB1 and ZSB17 strains on 13 selected growth and yield-related attributes, among all the treatments, the highest biological yield (q/ha) was observed in treatment T5 (143.82 ± 5.65 q/ha) which were a mixture of 100% RDF, ZSB1 isolates and $ZnSO_4$. The maize plant growth and production have been significantly increased through seed bacterization with ZSB isolates. Hussain et al. (2015) recorded an increase in plant growth attributes primarily shoot length, root length, shoot fresh and dry biomass, and root fresh and dry biomass when Zn solubilizing *Bacillus* sp. (AZ6) was inoculated under field conditions. Sarathambal et al. (2010) have demonstrated that the dry weight of the maize is increased compared with control by the inoculation of zinc solubilizing *Gluconacetobacter diazotrophicus*. An experiment conducted by Goteti et al. (2013) in which they revealed that seed bacterization with zinc solubilizing PGP bacteria facilitates the growth of plant height (root and height of the shoot); leaf area; and dry mass.

The results of the study on the effect of ZSB isolates on zinc translocation index (ZTI) in maize plant are presented in Table 4. Zinc translocation index is used in this study as a similar notion to the translocation factor (TF) that can be viewed as the ratio of an element in a plant's shoots and roots (Upadhyay et al., 2021). The maize plant showed the highest ZTI (55.21%) in treatment T5 (ZSB1 + 100% RDF + $ZnSO_4$), followed by treatment T12 (ZSB17 + 100% RDF + $ZnSO_4$; 53.4%). This clearly shows ZSB isolates have role in translocation of Zn toward maize grains and similar finding was earlier

reported by Goteti et al. (2013) and Omara et al. (2016). In comparison to the control, the introduction of *B. aryabhattai* isolates to wheat and soybean crops dramatically boosted Zn uptake as well as shoot and seed weight (He et al., 2010). In addition to synergistic impact on plants' growth and yield, ZSBs have a strong capacity to enhance the Zn content of cereals which ultimately improves human health and immunity (Abaid-Ullah et al., 2015; Wang et al., 2014). Krithika and Balachandar (2016) reported that ZSB up-regulated the expression of Zn-regulated transporters and iron (Fe)-regulated transporter-like protein (ZIP) genes in rice suggested its important role in zinc fertilization and fortification. Uptake of micronutrients (Zn) by the plants from soil is a mutually dependent process (Bouain et al., 2014). Using microbial tools to enhance the availability of soil Zn to crop plants is one of the sustainable ways of reducing the Zn deficiency and improving Zn content of food crops grain in zinc deficient soils (Sirohi et al., 2015). Furthermore, such microbial tools will improve the zinc deficient soil and restore them to healthy soil by improving available zinc in soil. The ZSB isolates from the present study can be used for development of liquid biofertilizers to improve zinc acquisition in different crop plants cultivated in southern Rajasthan based on dedicated field studies.

5 | CONCLUSION

The primary issue that inhibits plant growth performance in degraded soil is the type of zinc that is not readily available to plants; zinc-deficient soil is frequently observed in the current research sites. Zn is a crucial micronutrient needed for healthy plant development and growth, and a deficiency does more than just harm human health and crop productivity. The findings of this research demonstrated that two distinct native bacteria, *Cupriavidus* sp. and *Pantoea agglomerans*, had the highest potential to solubilize insoluble zinc in the form of zinc that was readily available and to promote maize growth at the field level. Both isolates (*Cupriavidus* sp. and *P. agglomerans*) demonstrated a variety of PGP properties and produced catalase and urease, both of which promoted plant development. *Cupriavidus* sp. and *P. agglomerans* increased the yield of maize by 19.01% and 17.64%, respectively, and improved Zn translocation toward grains. We conclude that the *Cupriavidus* sp. and *P. agglomerans*, considerably improved soil health, maize crop production, and both unique strains could play a spectacular and promising role in bio-fertilizer technology.

AUTHOR'S CONTRIBUTION

Devendra Jain designed the research. Aradhana Sukhwal performed the experiments. Vimal Sharma interpreted the data. Gajanand Jat performed soil and AAS analysis. Aradhana Sukhwal performed HPLC and GCMS studies. Devendra Jain and Sudhir K. Udpadhay wrote the manuscript. All authors reviewed the manuscript.

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CONFLICT OF INTEREST STATEMENT

No potential conflict of interest was reported by the authors.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

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RESEARCH ARTICLE

Comparative evaluation of native *Trichoderma* species from groundnut rhizosphere against stem rot caused by *Sclerotium rolfsii* Sacc.

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Abstract

Sclerotium rolfsii Sacc. is one of the important soil borne pathogen causing stem rot of groundnut prevalent in all growing area worldwide. The present study aimed on the identification of native *Trichoderma* isolates, and its efficacy against the stem rot pathogen in groundnut at field level. Thirty-five isolates of *Trichoderma* spp. isolated from the groundnut rhizosphere were comparatively evaluated for their biocontrol potential against *S. rolfsii* Sacc. and growth promoting traits in groundnut. The morphological studies of the 35 isolates were supported molecularly by amplifying of ITS region and classified into four species namely, *T. asperellum*, *T. citrinoviride, T. longibrachiatum* and *T. harzianum* which were further subjected to biocontrol efficacy tests. The highly efficient representative isolates namely, *T. harzianum* Thar23, *T. asperellum* Tasp49, *T. longibrachiatum* Tlongi5 and *T. citrinoviride* Tcitri2 were evaluated to produce lytic enzymes and growth promoting traits. The comparative study of these isolates revealed that, *T. harzianum* Thar23 produced significant ($P < 0.05$) amount of lytic enzymes viz., chitinase (31.36 U/ml), β 1, 3 glucanase (4.1 U/ml) and protease (2.76 U/ml). *T. harzianum* Thar23 promotes plant growth traits namely germination efficacy (31.48%), increase in the shoot length (42%) and root length (42.43%), improved vigor index, and increased relative water content (25.56%). Soil application, seed treatment and drenching with the powder formulation of Thar23 in field for the years 2019 and 2020 significantly ($P < 0.05$) reduced stem rot disease incidence to 59.45% and 53.79% and increased pod yield to 2.85 t/ha and 2.68 t/ha respectively. *T. harzianum* isolate Thar23 will help the groundnut growers for eco-friendly management of stem rot disease and increased yield.

Keywords Groundnut · Lytic enzymes · *S. rolfsii* · Stem rot · *Trichoderma* spp.

Introduction

Groundnut (*Arachis hypogaea* L.) is an important food and oil seed crop due to its high protein and oil content. Several biotic and abiotic factors are responsible for dismal productivity. Diseases like stem rot, collar rot, root rot, leaf spot, bud necrosis, etc., are critical. Stem rot is also known as sclerotium blight caused by soil borne fungi *S. rolfsii* causes yield loss over 20–25 percent (Annual Report 2015–16). Under warm and high moisture conditions, white mycelium spread over the plant debris, soil and infect the host. The dark brown sclerotia of the pathogen are hard, spherical and 0.5–1.5 mm in size often found in the infected are of host and soil (Aycock 1966). Though fungicides are effective against pathogens, but they cause adverse effect on the environment thus can be replaced by biocontrol agents.

Trichoderma spp. (Teleomorph: *Hypocrea*) is an omnipresent ascomycetous fungus known for its biocontrol and industrial properties. This fungi were named *Trichoderma* in 1794 (Persoon 1794) and years later in 1865, the sexual stage Hypocrea species was suggested (Tulasne and Tulasne 1865). Diverse species of *Trichoderma* namely, *T. harzianum, T. asperellum, T. viride, T. virens, T. hamatum*

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and *T. atroviride* have been reported as biocontrol agents. *T. reesei, T. parareesei* and *T. longibracheatum* are known for industrial enzyme production. *Trichoderma* species are widely present in the soil rhizosphere and documented for symbiotic relationship with the host roots. Due to the importance of the application of *Trichoderma* spp. as biocontrol agent in field condition, it is necessary to explore its biogeography. There are different studies conducted by the researchers to decipher the diversity of the native *Trichoderma* spp. and its application against major plant pathogens at national (Kumar et al. 2012; Agrawal and Kotasthane 2012; Devi et al. 2021; Manzar et al. 2021; Jambhulkar et al. 2022) and global (Li et al. 2016; Boat et al. 2020; Ma et al. 2020; Nofal et al. 2021). However, there is a need to explore the diversity of the native species at groundnut growing area of Jaipur, a semi-arid eastern plain zone of Rajasthan (Agro-climatic Zone- III-A), India.

There are various biocontrol mechanisms viz., mycoparasitism, antibiosis, induced systemic resistance in *Trichoderma* spp. and also known for production of many lytic enzymes viz., chitinases, glucanases, xylanase and proteases etc., as their primary weapons against the fungal pathogens (Sharma et al. 2014) and induce the systemic defence response by activating defence enzymes like peroxidases (PODs), polyphenol oxidases (PPO) and phenylalanine ammonia lyase (PAL) (Malolepsza et al. 2017). Plant growth promotion is crucial component of *Trichoderma* spp. which helps in improvement of plant growth in terms of increased plant biomass, root and shoot length and grain yield. *Trichoderma* colonizes fully on root tissues and triggers various mechanisms which induce plant growth promotion, facilitate nutrient uptake, induce plant defence mechanisms, helps in rhizosphere construction, increase carbohydrate metabolism, induce of phytohormones, root exudates and photosynthesis in host (Sallam et al. 2019). Among the genus of *Trichoderma* spp., *T. harzianum* is the most researched biocontrol species followed by others such as *T. viride*, *T. asperellum*, *T. hamatum*, *T. virens* and *T. koningii* (Keswani et al. 2014). Species like *T. longibrachiatum* and *T. citrinoviride* needs to be studied for its biocontrol and plant growth promoting capabilities. Therefore, the comparative evaluation of biocontrol efficacy and plant growth promoting traits of native isolates of *Trichoderma* spp. will be helpful in the characterization of biocontrol control agents and potential strains can be utilized at field conditions.

In the present study, we have isolated and characterized native isolates of *Trichoderma* from groundnut rhizosphere and potent isolates were comparatively evaluated to assess biocontrol and plant growth promoting potential against groundnut stem rot pathogen *S. rolfsii* under field conditions.

Materials and methods

Collection and isolation of *Trichoderma* isolates

The 60 rhizospheric soil samples were collected from groundnut growing areas of Jaipur (Agro-climatic Zone- III-A), a semi-arid eastern plain zone of Rajasthan, India. The longitude and latitude of collection locations were recorded and are given Table 1. For the isolation of *Trichoderma* spp., the rhizospheric soil samples were serially diluted on *Trichoderma* selective medium (TSM) (Elad et al. 1981) and incubated at 28 °C ± 1 °C for 4 days. The newly emerging mycelia of fungal colonies were subcultured to fresh potato dextrose agar (PDA) plates and incubated at 28 °C ± 1 °C for 7 days and maintained in potato dextrose agar (PDA) slants at 4 °C for further use in experiment. Culture of *S. rolfsii* was available at Department of Plant Pathology, SKN Agriculture University, Jobner- Jaipur.

Identification of *Trichoderma* isolates

Morphological identification

The purified 35 isolates were identified based on the different morphological characters viz., cultural characters like colour, growth and texture, microscopic features branching of conidiophores, phialides disposition, size and shape of conidia were identified based on the Rifai (1969), Bissett (1984) and Samuels et al. (1999).

Molecular identification

Actively growing *Trichoderma* isolates (5 mm disc) were inoculated into 50 mL potato dextrose broth (PDB) (HIMEDIA Labs, India) and incubated at 28 ± 2 °C for 5–6 days at 180 rpm. The fungal mycelia were harvested using Whatman No. 1 filter paper and washed three times with sterile distilled water. Collected mycelium was grounded finely with liquid nitrogen and stored at − 80 °C till further use. Cetyltrimethyl ammonium bromide (CTAB) method was followed for total fungal DNA extraction (Culling 1992). The internal transcribed spacer (ITS) region was amplified by using universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′)/ITS4 (5′-TCCTCCGCTTATTGATATGC3′) (White et al. 1990).

PCR reaction mixture was prepared in the final volume of 25 µl containing 2.5 µl of 10X PCR buffer with $MgCl_2$, 1 µl of forward and reverse primer each (10 pM), 0.5 µl of 10 mM dNTP's, 0.5 µl of DyNAzyme II DNA Polymerase (2 U/µl), 2 µl of genomic DNA (50 ng/µl) and 17.5 µl of Molecular biology grade water and amplified by following

Table 1 List of *Trichoderma* isolates along with NCBI accession numbers and morphological specifications

S. no.	Isolate code	Location	Latitude and longitude	NCBI accession number	*Trichoderma* spp.	Morphological characteristics
1	Tasp1	Jobner, Jaipur	26° 58′ 33.6″ N 75° 22′ 10.7″ E	KT426888	Group I—*Trichoderma asperellum*	Colony: dark green Conidiophores: regularly branched, with lateral branches and paired Phialides: whorls of 2–4 phialides, straight, slightly wider in middle than base and ampulliform Conidia: globose to subglobose
2	Tasp2	Durgapura, Jaipur	26° 50′ 41.0″ N 75° 46′ 52.5″ E	KT426889		
3	Tasp3	Bagru, Jaipur	26° 49′ 02.3″ N 75° 33′ 09.8″ E	KT426890		
4	Tasp4	Samod Jaipur	27° 14′ 01.5″ N 75° 46′ 53.1″ E	KT426891		
5	Tasp5	Sambhar, Jaipur	27° 00′ 12.3″ N 75° 11′ 24.7″ E	KT426892		
6	Tasp6	Chaksu Jaipur	26° 35′ 55.6″ N 75° 56′ 27.1″ E	KU170973		
7	Tasp46	Jobner, Jaipur	26° 58′ 59.3″ N 75° 32′ 35.1″ E	MT065825		
8	Tasp47	Chaksu Jaipur	26° 36′ 14.7″ N 75° 55′ 33.1″ E	MT065826		
9	Tasp48	Durgapura, Jaipur	26° 55′ 51.8″ N 75° 45′ 14.2″ E	MT065827		
10	Tasp49	Samod Jaipur	27° 12′ 32.6″ N 75° 46′ 57.5″ E	MT065828		
11	Tasp50	Jobner, Jaipur	26° 59′ 54.9″ N 75° 22′ 06.5″ E	MT065829		
12	Tasp51	Bagru, Jaipur	26° 50′ 08.7″ N 75° 33′ 19.1″ E	MT065830		
13	Thar1	Durgapura, Jaipur	26° 55′ 35.0″ N 75° 42′ 06.1″ E	KT426893	Group II—*Trichoderma harzianum*	Colony: whitish green to pale green Conidiophores: flexuous, branches almost right angled Phialides: whorls of 2–6, ampulliform to laginiform, subulate, short, skittle-shaped, narrower at the base Conidia: globose to subglobose to short obovoid
14	Thar2	Samod Jaipur	27° 11′ 08.5″ N 75° 46′ 59.9″ E	KT426894		
15	Thar3	Jobner, Jaipur	26° 57′ 56.3″ N 75° 22′ 35.9″ E	KT426895		
16	Thar4	Durgapura, Jaipur	26° 50′ 30.0″ N 75° 47′ 00.2″ E	KT426896		
17	Thar5	Jobner, Jaipur	26° 58′ 46.2″ N 75° 22′ 21.1″ E	KT426897		
18	Thar20	Samod Jaipur	27° 11′ 08.5″ N 75° 46′ 59.9″ E	MT065754		
19	Thar21	Durgapura, Jaipur	26° 50′ 46.1″ N 75° 46′ 46.4″ E	MT065755		
20	Thar22	Bagru, Jaipur	26° 50′ 08.7″ N 75° 33′ 19.1″ E	MT065756		
21	Thar23	Jobner, Jaipur	26° 58′ 33.6″ N 75° 22′ 10.7″ E	MT065757		
22	Thar24	Sambhar, Jaipur	26° 52′ 26.2″ N 75° 07′ 38.4″ E	MT065758		
23	Thar25	Chaksu Jaipur	26° 35′ 55.6″ N 75° 56′ 27.1″ E	MT065759		

Table 1 (continued)

S. no.	Isolate code	Location	Latitude and longitude	NCBI accession number	*Trichoderma* spp.	Morphological characteristics
24	Tlongi1	Jobner, Jaipur	26° 93′ 21.6″ N75° 37′ 73.1″ E	KT426898	Group III—*Trichoderma longibrachiatum*	Colony: dark olive green with yellow tinge Conidiophores: long main branches produce only a few side short branches Phialides: laginiform or bottle shaped Conidia: sub-cylindrical with distinct truncate base
25	Tlongi2	Bagru, Jaipur	26° 49′ 02.3″ N 75° 33′ 09.8″ E	KT426899		
26	Tlongi3	Chaksu Jaipur	26° 36′ 14.7″ N 75° 55′ 33.1″ E	KT426900		
27	Tlongi4	Samod Jaipur	27° 12′ 32.7″ N 75° 51′ 31.3″ E	KT426901		
28	Tlongi5	Sambhar, Jaipur	27° 00′ 12.3″ N 75° 11′ 24.7″ E	KT426902		
29	Tlongi25	Durgapura, Jaipur	26° 55′ 35.0″ N 75° 42′ 06.1″ E	MT052706		
30	Tcitri1	Bagru, Jaipur	26° 48′ 20.4″ N 75° 33′ 05.6″ E	MT065795	Group IV—*Trichoderma citrinoviride*	Colony: dusky yellowish green Conidiophores: main branches long and relatively straight Phialides: more elongate, lageniform or narrowly shaped Conidia: less ellipsoidal, apex broadly rounded
31	Tcitri2	Chaksu Jaipur	26° 34′ 58.2″ N 75° 59′ 48.2″ E	MT065796		
32	Tcitri3	Samod Jaipur	27° 12′ 32.7″ N 75° 51′ 31.3″ E	MT065797		
33	Tcitri4	Sambhar, Jaipur	27° 01′ 58.6″ N 75° 18′ 48.0″ E	MT065798		
34	Tcitri5	Jobner, Jaipur	26° 59′ 40.4″ N 75° 20′ 49.4″ E	MT065799		
35	Tcitri6	Durgapura, Jaipur	26° 50′ 24.1″ N 75° 46′ 51.2″ E	MT065800		

protocol: initial denaturation for 1 min at 95 °C, 30 cycles of denaturation for 30 s at 95 °C, primer annealing for 1 min at 60 °C, extension at 72 °C for 1 min and a final extension period for 7 min at 72 °C. The amplified PCR products were electrophoretically separated using 1.2% agarose gel in 1X TAE buffer at 80 V for 1 h. Amplified PCR fragments were visualized in UV light and gel documented. The desired amplified products were gel eluted (GeneJET Gel Extraction Kit, Thermo Scientific™, USA) and sequenced through the Sanger sequencing method (Eurofins Pvt. Ltd). The sequence contig was prepared using CAP3 sequence assembly program and aligned sequence were confirmed with nBLAST (www.ncbi.nlm.nih.gov/BLAST) and submitted to NCBI (http://www.ncbi.nlm.nih.gov/).

Phylogenetic analysis

The phylogenetic tree was constructed by aligning the generated sequences using ClustalW multiple sequence alignment program (Thompson et al. 1994) and MEGA7 software program (Kumar et al. 2016). Maximum Composite Likelihood (MCL) method was used to estimate the pairwise distances and bootstrap method was used to study the nodal robustness with a replication of 1000. The Kimura 2-parameter distance model (Kimura 1980) was used for the construction of maximum-likelihood (ML) tree (Kumar et al. 2016).

Screening for antagonistic activity of *Trichoderma* isolates

Antagonistic activity of different 35*Trichoderma* isolates against groundnut stem rot pathogen *S. rolfsii* was done by dual culture plate method (Dennis and Webster 1971). Seven days old actively growing mycelial disc (5 mm) of *Trichoderma* isolates and *S. rolfsii* were placed on PDA plates opposite from the periphery and plates without *Trichoderma* served as a control and plates were incubated at 28 ± 2 °C for 5–7 days. The percentage of inhibition was calculated by following formula

$$\text{Percentage of inhibition (PI)} = \frac{C-T}{C} \times 100$$

where 'C' is the radial growth of pathogen in the control PDA plate in cm and 'T' is the radial growth pathogen in test plate in cm.

The antagonism level of these isolates was evaluated according to Bell et al. (1982). *Trichoderma* isolates with significant antagonistic potential against *S. rolfsii* were

evaluated for production of lytic enzymes and plant growth promoting traits in groundnut.

Lytic enzymes assay of selected isolates of four *Trichoderma* spp.

Preparation of cell-free culture filtrate

The cell-free culture filtrate from selected isolates *T. harzianum* Thar23, *T. asperellum* Tasp49, *T. longibrachiatum* Tlongi5 and *T. citrinoviride* Tcitri2were prepared using freeze-dried mycelia of *S. rolfsii* as a sole carbon source. Actively grown mycelial mat of *S. rolfsii* was harvested from 7 days old PDB broth and homogenized by using liquid nitrogen. The freeze dried pathogen mycelial powder was stored at − 20 °C. A 5 mm mycelial disc of actively growing selected *Trichoderma* isolates was inoculated in autoclaved 250 ml of minimal synthetic broth (MSB) containing (g/l) $FeSO_4$-0.01, $MnSO_4$-0.01, $ZnSO_4$- 0.01, KCl-0.5, $MgSO_4$-0.5, K_2HPO_4-1.0, $NaNO_3$-3.0; pH 5.5 amended with 1% freeze dried mycelia of *S. rolfsii* and flasks were kept at 28 ± 2 °C at 180 rpm and filtered through Whatman no. 1 filter paper at different time interval from day 1 to 10.

Estimation of chitinase (EC 3.2.1.14)

Dinitrosalicylic acid (DNSA) method was used to estimate the chitinase production from *Trichoderma* isolates. One millilitre of culture filtrate with 0.5 ml of colloidal chitin and 0.5 ml of 1 M sodium acetate buffer was mixed and incubated at 40 °C for 6 h and centrifuged at 12,000 rpm for 5 min at 4 °C. One millilitre of supernatant was mixed with 0.5 ml of DNSA in 1 M NaOH and 0.1 ml of 10 M NaOH and kept at 100 °C for 5 min. The assay mixture was recorded spectrophotometrically at 582 nm and N-acetylglucosamine (GlcNAc) was used as standard. Specific chitinolytic activity was defined as unit of GlcNAc released by 1 ml of enzyme solution under assay conditions.

Estimation of β-1,3-glucanase (EC 3.2.1.39)

β-1,3-Glucanase activity was determined using laminarin as a substrate. The assay mixture contains 0.5 ml of culture filtrate with 1 ml of laminarin in 50 mM acetate buffer (pH 4.8) and was incubated at 50 °C for 10 min. One ml of dinitrosalicylic acid was added to the reaction mixture and kept at 95 °C for 5 min and total amount of reducing sugar was recorded at 540 nm. One unit of β-1,3-glucanase activity was defined as the amount of enzyme required to release one µmol of reducing sugar per minute.

Estimation of protease (EC 3.4.21.4)

Protease activity was determined using 1% casein as substrate in 50 mM phosphate buffer (pH 7.0) was denatured at 100 °C for 15 min in the water bath. The reaction mixture containing 1 ml of casein substrate was added with 3 ml of 10% tricholoroacetic acid (TCA) and kept at 4 °C for 1 h. This mixture was centrifuged at 8000 rpm for 15 min at 4 °C, and supernatant was recorded at 280 nm. One unit of protease activity was defined as the amount of enzyme solution equivalent to release 1 µmol of tyrosine under assay conditions.

Plant growth promoting traits of selected isolates of four *Trichoderma* spp. in groundnut

The plant growth promoting ability of the selected *Trichoderma* isolates in groundnut (RG-510 Spreading variety) was studied under pot conditions. Groundnut seeds were treated with spore suspensions of each selected *Trichoderma* isolates containing 2×10^8spores $ml^{-1}$and were soaked for one hour. Spore suspensions from selected isolates were prepared from PDA plates containing 7 days old cultures of *Trichoderma* by scraping gently on the surface of the plates with sterile distilled water containing 0.01% Tween 20 and filtered through two layers of sterile muslin cloth. The spore concentration was adjusted with the aid of haemocytometer.

Efficacy on seed germination, root, and shoot length and relative water content (RWC)

The germination efficacy of selected *Trichoderma* isolates in groundnut seeds was studied by treating with *Trichoderma* spore suspension (2×10^8 spores ml^{-1}) and transferred to respective pots containing sterile soil along with farm yard manure (FYM) in 10:1 ratio. Seeds treated with sterile water served as control. After 10 days, the number of germinated seedlings in each replication was counted and the germination was calculated and expressed by using the following formula

$$\text{Germination percentage (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Groundnut plants (30 days old) were harvested from each treatment and washed three times with sterile distilled water. The root and shoot length were observed and based on the root and shoot length with germination percentage, the vigour index was calculated by using formula given by Abdul Baki and Anderson (1973).

$$\text{Vigour Index (VI)} = (\text{Mean shoot length} + \text{Mean root length}) \times \text{Germination (\%)}$$

To determine relative water content, the harvested plants were air dried and weighed (fresh weight). For dry weight, the plants were kept in hot air oven at 100 °C for 20 min, and then kept at 80 °C for 24 h at oven then weighed and recorded (Tian et al. 2015). Each control and treatment were repeated three times. The following formula was used to determine RWC of shoots and roots.

$$\text{RWC (\%)} = \frac{\text{FW - DW}}{\text{FW}} \times 100$$

where, RWC is relative water content, FW: fresh weight, and DW: dry weight.

Groundnut stem rot management by application of selected isolates of four *Trichoderma* spp. under field conditions

The field experiment was conducted in the randomized block design with three replications in the kharif season of 2019 and 2020 at Agronomy Farm, S.K.N. College of Agriculture, Jobner situated 260 05′ N-latitude and 750 28′ E-longitudes and at an altitude of 427 m above mean sea level in Jaipur district of Rajasthan. The region falls in agroclimatic zone III-a (semi-arid eastern plain), and variety RG 510 was used for both experimental years. The seeds were treated with talc-based bioformulation of different *Trichoderma* isolates at 8 g/kg. The spore concentration of the bioformulation was maintained 2×10^8 CFU/g. The treatment schedule is as follows.

T1—Soil application with *T. asperellum* Tasp49 enriched FYM (10: 200) + seed treatment with *T. asperellum* Tasp49 at 8 g/kg seeds + drenching with *T. asperellum* Tasp49 at 8 ml/l at 40 days after sowing.

T2—Soil application with *T. harzianum* Thar23 enriched FYM (10: 200) + seed treatment with *T. harzianum* Thar23 at 8 g/kg seeds + drenching with *T. harzianum* Thar23 at 8 ml/l at 40 days after sowing.

T3—Soil application with *T. longibrachiatum* Tlongi5 enriched FYM (10: 200) + seed treatment with *T. longibrachiatum* Tlongi5 at 8 g/kg seeds + drenching with *T. longibrachiatum* Tlongi5 at 8 ml/l at 40 days after sowing.

T4—Soil application with *T. citrinoviride* Tcitri2 enriched FYM (10: 200) + seed treatment with *T. citrinoviride* Tcitri2 at 8 g/kg seeds + drenching with *T. citrinoviride* Tcitri2 at 8 ml/l at 40 days after sowing.

T5—Untreated control.

Disease incidence was monitored on a weekly basis by observation of symptoms and was calculated by the following formula

$$\text{Disease incidence (DI) (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Shelling of the well dried 100 g pods from each treatment was done and recorded weight of kernels and the shelling percentage was calculated by following formula

$$\text{Shelling percentage} = \frac{\text{Kernel weight}}{\text{Pod weight}} \times 100$$

The pod yield was calculated from each treatment separately after threshing, winnowing, and cleaning the produce was weighed and converted in terms of Tones/ha.

Statistical analysis

The normality of the data was checked and found that data are treatment-wise normally distributed. All the treatments replicated thrice in a completely randomized design and the descriptive statistics of the data are presented as mean value ± SD. Significance of mycelial growth inhibition, enzyme production and growth promotion were tested by a one-way analysis of variance (ANOVA). The data were analysed by ANOVA using R-programming language and treatment means were compared using Fisher's Protected LSD test at p = 0.05 (Gomez and Gomez 1984).

Results

Morphological characteristics of *Trichoderma* isolates

Thirty-five isolates of *Trichoderma* spp. were collected from the rhizospheric soil of groundnut growing area of Jaipur District (26.9706° N, 75.3791° E) of Rajasthan, India, which were further morphologically characterized through microscopic studies. Based on morphological features the isolates were classified into four groups I *T. asperellum*, group II *T. harzianum*, group III *T. longibrachiatum* and group IV *T. citrinoviride* (Table 1).The group I consisted of 12 isolates of *T. asperellum* showed dark green and compact colonies on PDA medium with the typically paired and regularly branched conidiophores(Table 1). The conidia were globose to sub-globose in shape with the size of 2.5–3 µm (Fig. 1). A total of 11 isolates of *T. harzianum* in grouped exhibited whitish green to pale green on PDA surface with short branched and irregular conidiophores at right angle. The shape of conidia was globose to sub-globose to short obovoid with size of 1.5–2 µm (Fig. 1). The 6 isolates of group III were yellowish green or dark olive green on PDA plates with short, branched conidiophores, laginiform or bottle shaped conidia on long main branches with the size of

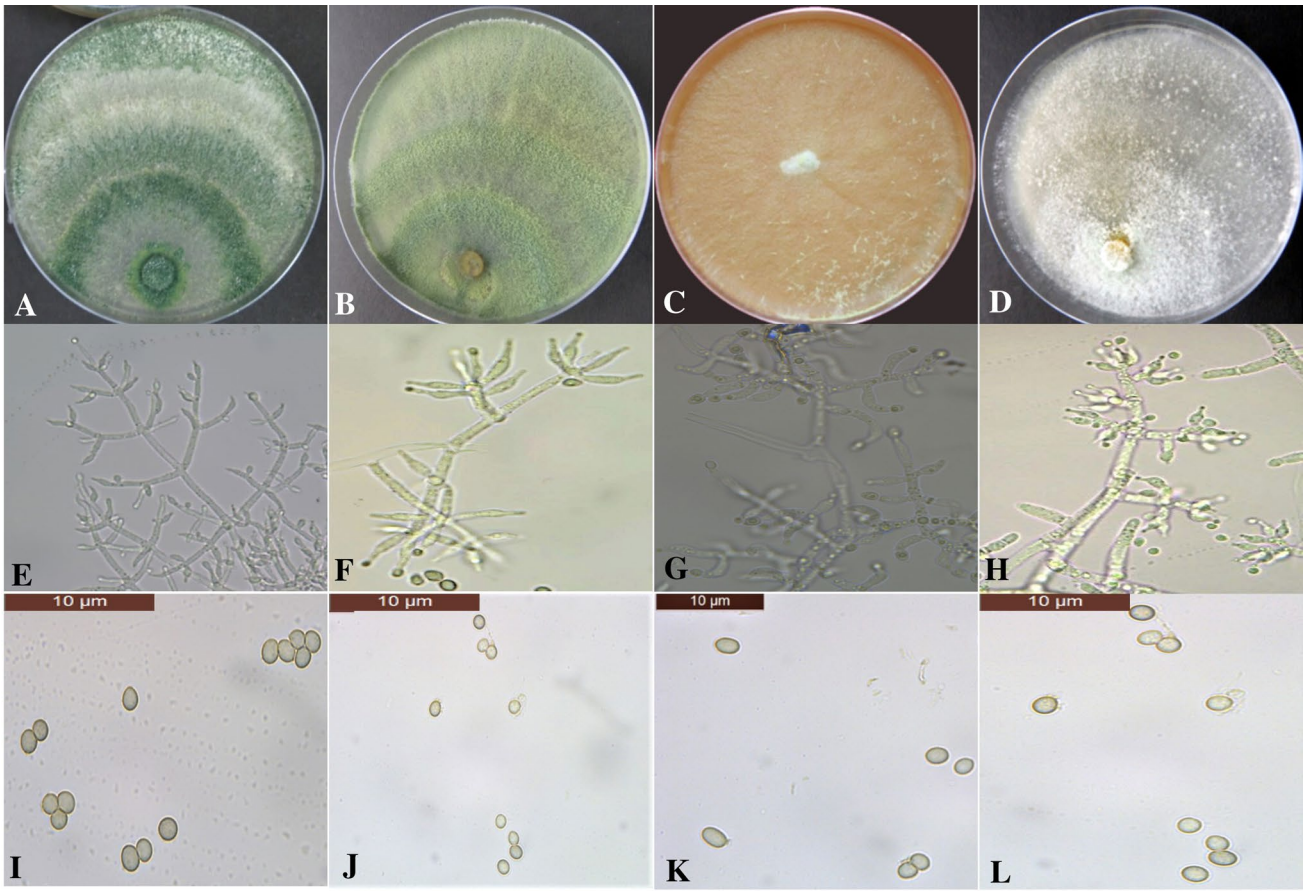

Fig. 1 PDA culture plates showing 7 days old representative isolates of *Trichoderma* spp. **a**, **e**, **i** Showing the growth on PDA, branching pattern of phialides and conidia of *T. asperellum* Tasp49. **b**, **f**, **j** Showing the growth on PDA, branching pattern of phialides and conidia of *T. harzianum* Thar23. **c, f, k** Showing growth on PDA, branching pattern of phialides and conidia of *T. longibrachiatum* Tlongi5. **d, h, l** Showing growth on PDA, branching pattern of phialides and conidia of *T. citrinoviride* Tcitri2 (scale bar 10 µm)

2–2.5 µm were classified as *T. longibrachiatum.* The group IV was classified as *T. citrinoviride* consisted of 6 isolates which showed dusky yellowish green colony on PDA with less ellipsoidal, broadly rounded apex conidia with size of 2–2.5 µm, with relatively straight long branched conidiophores, relatively elongate, lageniform or narrowly shaped phialides (Fig. 1).

Molecular characterization of *Trichoderma* isolates and phylogenetic analysis

A single amplified product around 550-650 bp of all 35 isolates of *Trichoderma* spp. were sequenced and confirmed with BLAST search tool and submitted to NCBI (Table 1). The BLAST analysis was differentiating at species level with homology percentage of 95–99%, and results obtained from phylogenetic analysis of ITS sequences showed that the 35 *Trichoderma* isolates can be separated into four different species with three distinct clades of *Trichoderma* namely (Fig. 2), the Pachybasium A clade (*T. asperellum*), the Harzianum clade (*T. harzianum*), and the Longibrachiatum clade (*T. longibrachiatum* and *T. citrinoviride*). The Pachybasium A clade consists of 12 isolates of *T. asperellum* with a bootstrap value of 98%, the clade Harzianum consisting of 11 isolates of *T. harzianum* supported by bootstrap value of 81%. The closely associated species both *T. longibrachiatum* (6 isolates) and *T. citrinoviride* (6 isolates), fall in the section Longibrachiatum clade with 96% bootstrap value indicating the close relationship of both species (Fig. 2).

Antagonistic activity of *Trichoderma* isolates against *S. rolfsii*

The antagonistic activity of 35 isolates of *Trichoderma* spp. against *S. rolfsii* was evaluated by dual culture assay. Two groups of *T. asperellum* and *T. harzianum* exhibited higher antagonistic activity with the range of 62% to 81.7% against *S. rolfsii*. Group III *T. longibrachiatum* and Group IV *T. citrinoviride* recorded moderate or lower mycelial inhibition from 51.43 to 67.5% (Fig. 3).The degree of antagonism was

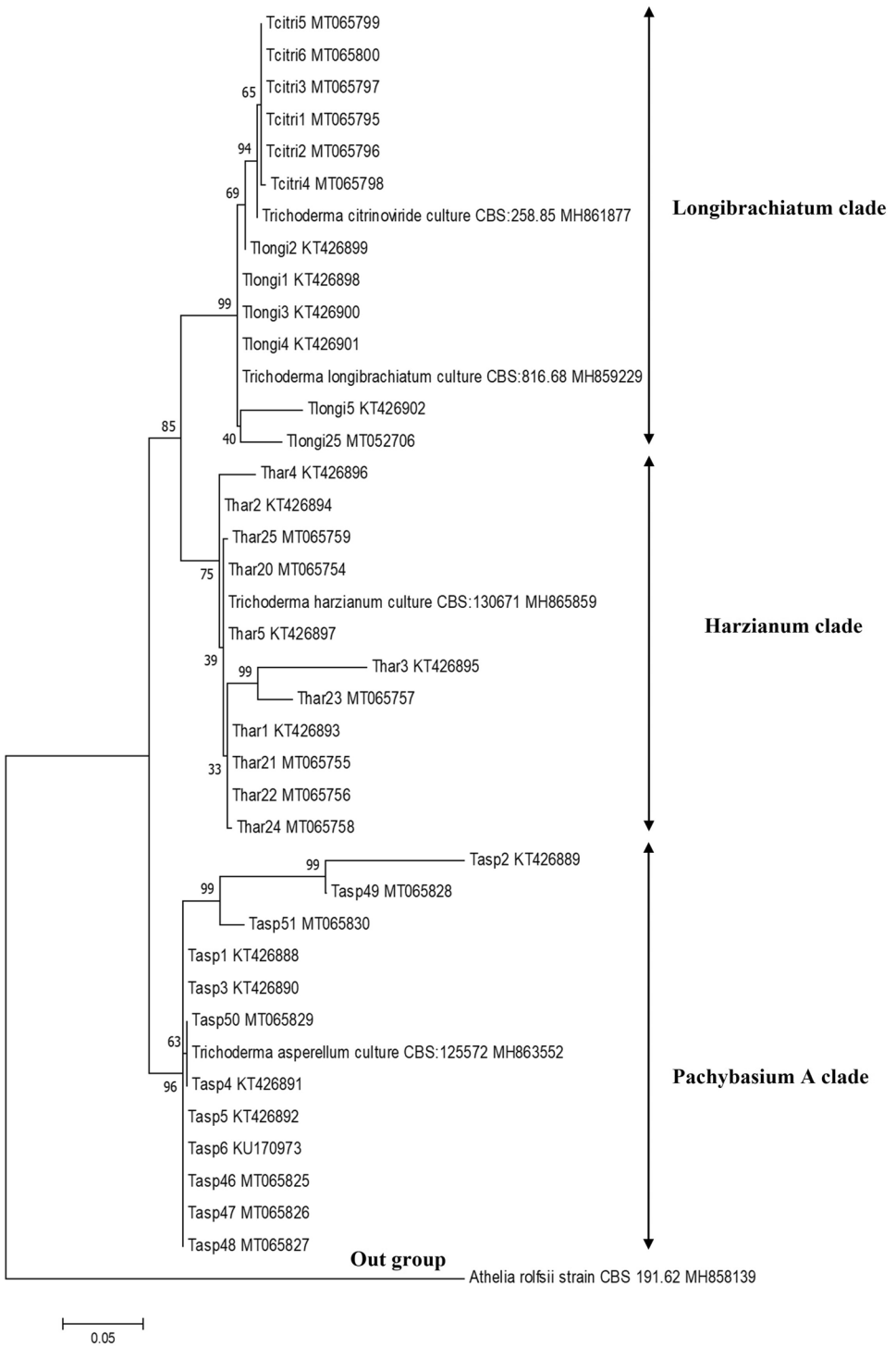

Fig. 2 Phylogenetic relationships of *Trichoderma* isolates inferred by analysis of ITS region and constructed using two parameter model implemented in the MEGA7 inferred by using the Maximum Likelihood method and Tamura-Nei model. Analysis was conducted in MEGA 7 and the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test

measured by the scale described by Bell et al. (1982). Isolates from *T. asperellum* group namely Tasp1, Tasp3, Tasp6, Tasp46 and Tasp49, from *T. harzianum* group namely, Thar1, Thar3, Thar4, Thar5, Thar20, Thar21, Thar22, Thar23, Thar24 and Thar25 exhibited class 1 level of antagonism, whereas isolates like Tasp2, Tasp4, Tasp5, Tasp47, Tasp48, Tasp50 and Tasp51 from *T. asperellum* group, one isolate from *T. harzianum* Thar2, some of the isolates from *T. longibrachiatum* group namely Tlongi1, Tlongi2, Tlongi4, Tlongi5and Tcitri2, Tcitri4 and Tcitri6 from *T. citrinoviride* group expressed the class 2 level of antagonism. Class 3 level of antagonism was observed in Tlongi3 and Tlongi25 from *T. longibrachiatum* and Tcitri1, Tcitri3 and Tcitri5 from *T. citrinoviride* against *S. rolfsii*. Among 35 isolates,

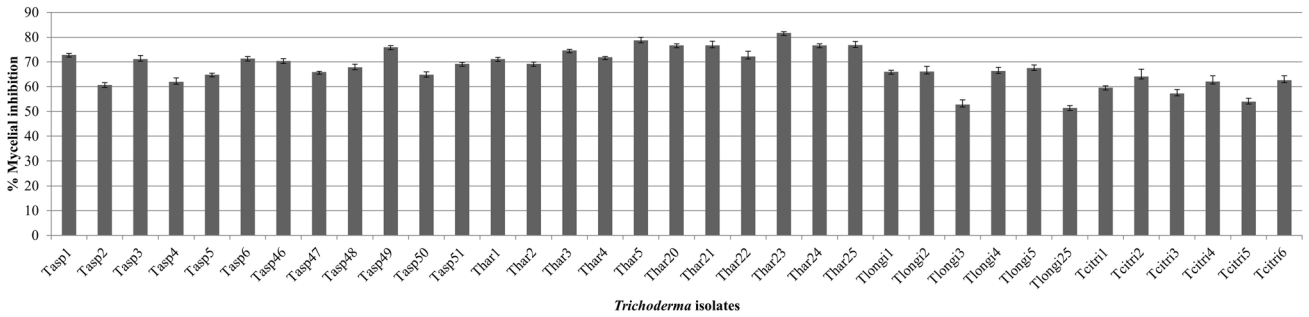

Fig. 3 Per cent mycelial inhibition of *S. rolfsi* by different *Trichoderma* isolates in dual culture assay. Treatment means were compared using Fisher's Protected LSD test (p = 0.05)

the potential isolate from each group namely *T. asperellum* Tasp49 from group I, *T. harzianum* Thar23 from group II, *T. longibrachiatum* Tlongi5 from group III and *T. citrinoviride* Tcitri2 from group IV were selected for the study of lytic enzyme production and plant growth promoting traits in groundnut.

Lytic enzymes assay of selected isolates of four *Trichoderma* spp.

The lytic enzymes like chitinase, β-1,3-glucanase and protease from selected *Trichoderma* isolates were studied by using freeze dried mycelia of *S. rolfsii* in MSB as a source of enzyme production. The enzyme activity from the isolates were gradually increased from day 1 to 7 for chitinase and day 1–6 for β-1,3-glucanase and protease and decreased after day 7. Among the selected isolates, *T. harzianum* Thar23 (31.36 U/ml) significantly produced higher amount of chitinase on day 7 followed by *T. asperellum* Tasp49 (25.26 U/ml) (Fig. 4). The other selected isolates *T. longibrachiatum* Tlongi5 (20.1 U/ml) and *T. citrinoviride* Tcitri2 (17.3 U/ml) exhibited lesser amount of chitinase enzyme activity as compared to other isolates (Fig. 4). Similarly, another lytic enzymes β -1, 3-glucanase and protease are also produced significantly higher 4.1 U/ml and 2.76 U/ml on day 6 by *T. harzianum* Thar23 followed by *T. asperellum* Tasp49 (2.6 U/ml and 2.13 U/ml). The other two selected isolates *T. longibrachiatum* Tlongi5 (1.33 U/ml and 1.16 U/ml) and *T. citrinoviride* Tcitri2 (0.8 U/ml and 0.83 U/ml) showed lesser production of these enzymes compared to other isolates (Fig. 4).

Plant growth promoting traits of selected isolates of four *Trichoderma* spp. in groundnut

The selected isolates were comparatively tested for their growth promoting ability in groundnut under greenhouse conditions. The seeds treated with *T. harzianum* Thar23 and *T. asperellum*Tasp49 significantly increased the germination efficacy to 31.48 and 24.47% and increased the shoot length by 42 and 21.44% and root length by 73.72 and 62.76% compared to control with vigour index of 3598.25 and 3030.65

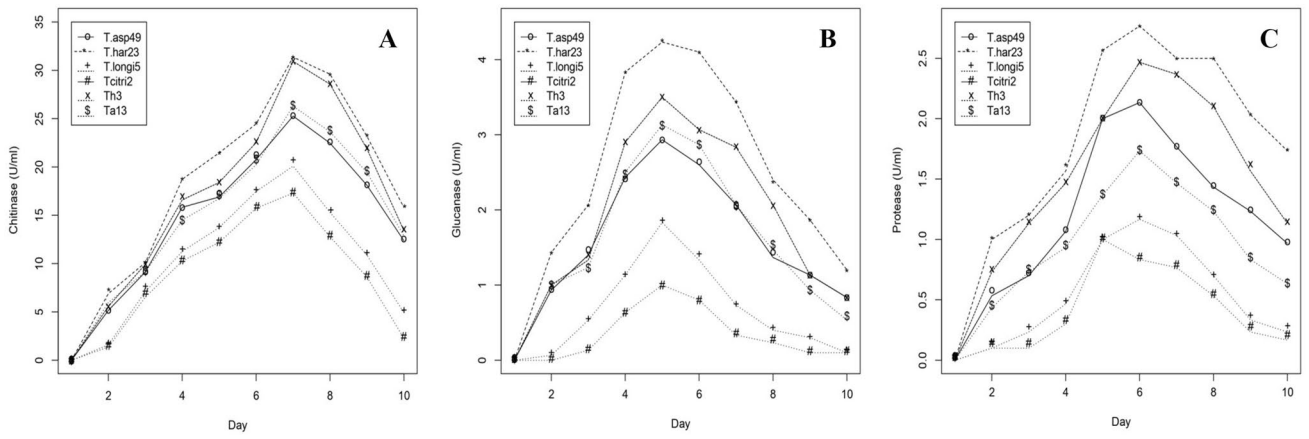

Fig. 4 Lytic enzymes **a** chitinase, **b** β-1,3-glucanase and **c** protease secretion (U/ml) from selected *Trichoderma* isolates at different time intervals

Table 2 Plant growth promoting efficacy of selected *Trichoderma* isolates in groundnut under glasshouse conditions

Trichoderma isolates	Shoot length (in cm)	Root length (in cm)	Germination (%)	Plant vigour index	Relative water content of shoot (%)	Relative water content of root (%)
Tasp49	22.27 ± 1.36^{c}	15.3 ± 0.1^{c}	80.67 ± 0.29^{d}	3030.65 ± 128.31^{d}	75.77 ± 0.02^{d}	77.73 ± 2.38^{d}
Thar23	26.03 ± 0.71^{d}	16.33 ± 0.23^{d}	85.2 ± 0.2^{e}	3598.25 ± 58.05^{e}	78.36 ± 0.27^{e}	81.23 ± 0.59^{e}
Tlongi5	20.36 ± 0.47^{b}	10.8 ± 0.26^{b}	70.33 ± 0.42^{c}	2191.98 ± 35.51^{c}	71.40 ± 0.84^{c}	70.47 ± 0.82^{c}
Tcitri2	19.4 ± 0.46^{ab}	9.37 ± 0.15^{a}	68.23 ± 0.25^{b}	1962.78 ± 20.64^{b}	67.55 ± 0.77^{b}	67.25 ± 1.02^{b}
Control	18.33 ± 0.21^{a}	9.4 ± 0.17^{a}	64.8 ± 0.529^{a}	1797.17 ± 26.98^{a}	66.46 ± 0.64^{a}	60.65 ± 2.71^{a}

Values given in the column are the average of three replications followed by standard deviation. The different small letters (a–e) superscripts within the column are significantly difference at $P \leq 0.05$

(Table 2) and increase in plant biomass in terms of the fresh and dry weight of shoot and root. The RWC of shoot and root treated with Thar23 shown higher (81.23%) than control (60.65%) (Table 2). However, the moderate effect on germination efficacy and plant growth promoting traits was observed in seed treatment with *T. citrinoviride* Tcitri2 as compared to other selected isolates (Table 2).

Groundnut stem rot management by application of selected isolates of four *Trichoderma* spp. under field conditions

Field experiments were conducted to evaluate the efficacy of native *Trichoderma* isolates on stem rot disease incidence, shelling percent and pod yield for the year of 2019 and 2020 kharif season. Soil application, seed treatment and drenching with *T. harzianum* Thar23 and *T. asperellum* Tasp49 significantly ($P < 0.05$) reduced stem rot disease incidence up to 59.45%, 52.01% in 2019 and 53.79%, 48.74% for the year of 2020 and increased pod yield in *T. harzianum* Thar23 (2.85 t/ha and 2.68 t/ha) and *T. asperellum* Tasp49 (2.62 t/ha and 2.55 t/ha) treated plots with increased shelling per cent (Table 3). However, treatment with *T. longibrachiatum* Tlongi5 and *T. citrinoviride* Tcitri2 shows moderate reduction in disease incidence up to 35.11%, 34.16% in 2019 and 34.16%, 33.21% in 2020 with pod yield of 2.13 t/ha, 2.05 t/ha in 2019 and 2.05 t/ha and 1.95 t/ha in 2020 (Table 3). The results obtained from field experiments that indicate the effect of application of *T. harzianum* Thar23 in improvement of the pod yield up to 51.59% and 38.58% during 2019 and 2020 kharif seasons, respectively.

Discussion

The present study was focused on morphological and molecular characterization, antagonistic ability, and plant growth promoting traits in the potential native *Trichoderma* isolates tested against stem rot pathogen *S. rolfsii* of groundnut. The isolates from rhizosphere soil of groundnut were collected and characterized based on the morphological characteristics to identify the species level by using the reference of Rifai (1969), Bissett (1984) and Samuels et al. (1999) and classified in to four groups, namely *T. asperellum* (12), *T. harzianum* (11), *T. longibrachiatum* (6) and *T. citrinoviride* (6). Morphological characterization of native *Trichoderma* isolates has earlier been taken up by several researchers (Rifai 1969; Bissett 1984; Pandian et al. 2016; Devi et al. 2021; Jambhulkar et al. 2022). In addition to supporting the reliability of morphological identification, isolates were further characterized molecularly by amplifying ITS region. Kullnig-Gradinger et al. (2002) described the multigene phylogeny approaches for the evolution of *Trichoderma* spp. by using the ITS1 and ITS2, 28S rDNA, mitSSU, *tef* 1 and ech42 genes. Indian researchers have widely surveyed in different locations of the country and have reported from the different geographical locations like New Delhi (Muthu and Sharma 2011), South Andaman Island (Kumar et al. 2012), Chhattisgarh (Agrawal and Kotasthane 2012), Manipur (Kamala et al. 2015) and Uttarakhand (Manzar et al. 2021), different states of India (Devi et al. 2021). The present study revealed the presence of diverse *Trichoderma* spp. in the rhizosphere of groundnut growing area of Jaipur District of Rajasthan. Mainly *T. asperellum* and *T. harzianum* were found to be dominant species with greater antagonistic potential against a wide range of phytopathogens. Till now, 375 species of *Trichoderma* spp. have been identified and their DNA barcoding information was deposited in the International Subcommission on Taxonomy of *Trichoderma* (ICTT) (http://www.trichoderma.info). The modern *Trichoderma* taxonomy methods help in the precise identification and reorganization of 50 new species of *Trichoderma* per year (Cai and Druzhinina 2021). Similar studies by Manzar et al. (2021) highlighted the phylogenetic relationship among the *Trichoderma* spp. based on the ITS and *tef-1α* sequences. Out of 20 isolates, nineteen isolates belonged to *T. asperellum* as compared to *T. harzianum* (one isolate). With the upcoming trend of development of potential native strains of *Trichoderma* spp. characterization

Table 3 Effect of soil application, seed treatment and drenching with selected *Trichoderma* isolates against *S. rolfsii* in groundnut under field conditions during 2019 and 2020 kharif season

Trichoderma isolates	Per cent disease incidence (PDI)		Reduction over control		Pods per plant		Shelling per cent		Pod yield (t/ha)	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Tasp49	18.66 ± 0.50^{c}	20.66 ± 0.66^{c}	52.01^{b}	48.74^{b}	25.33 ± 0.57^{b}	29.00 ± 1.00^{a}	61.43 ± 0.37^{b}	58.76 ± 0.51^{b}	2.62 ± 0.03^{b}	2.55 ± 0.04^{a}
Thar23	15.76 ± 0.66^{d}	18.63 ± 0.51^{d}	59.45^{a}	53.79^{a}	31.00 ± 1.00^{a}	22.33 ± 1.15^{b}	66.06 ± 0.15^{a}	62.16 ± 0.25^{a}	2.85 ± 0.04^{a}	2.68 ± 0.02^{a}
Tlongi5	25.23 ± 0.20^{b}	26.46 ± 0.45^{b}	35.11^{c}	34.37^{c}	20.00 ± 1.00^{c}	15.66 ± 0.05^{c}	54.93 ± 0.56^{c}	51.00 ± 0.20^{c}	2.13 ± 0.03^{c}	2.07 ± 0.05^{b}
Tcitri2	25.6 ± 0.43^{b}	26.93 ± 0.47^{b}	34.16^{c}	33.21^{c}	19.33 ± 1.15^{c}	16.00 ± 0.00^{c}	54.66 ± 0.23^{c}	50.50 ± 0.45^{c}	2.05 ± 0.03^{c}	$1.95 \pm 0.03b^{c}$
Control	38.9 ± 0.65^{a}	40.33 ± 0.41^{a}	–	–	18.66 ± 0.57^{c}	15.33 ± 0.57^{c}	50.7 ± 0.41^{d}	48.76 ± 0.23^{d}	1.88 ± 0.03^{d}	1.84 ± 0.43^{c}

Values given in the column are the average of three replications followed by standard deviation. The different small letters (a–e) superscripts within the column are significantly difference at $P \leq 0.05$

through molecular and morphological tools have become very important step in research.

To further utilize the native strains for biological control, antagonism tests are required to understand the mechanism under in vitro and in vivo conditions. The antagonistic ability of the *Trichoderma* isolates was tested against *S. rolfsii* showed significant reduction in the mycelial growth of pathogen. Significant variation was observed in the isolates from *T. asperellum* and *T. harzianum* while *T. longibrachiatum* and *T. citrinoviride* exhibited moderate efficacy. The CWDEs are specialized group (glycosyl-hydrolases, oxidoreductases, lyases, and esterases) of enzymes produced by *Trichoderma* spp. which are key component against wide range of phytopathogens. Recently Kaur et al. 2021 reported purified proteins both endochitinase and β-1,3-glucanase from *T. viride* isolate T1#3 degrade the hyphae of *R. solani* causing sheath blight in rice. Several research findings stated that the genus of *Trichoderma* is known to produce CDWs like chitinase, β-1,3-glucanase and protease are playing key role in the suppression of the growth of major soil borne pathogens (Guigon-Lopez et al. 2015; Li et al. 2016; Elamathi et al. 2018; Boat et al. 2020; Macena et al.2020). In recent years, green synthesis of nanoparticles by these species made an impact in the agricultural and food sector due to the secretion of bioactive enzymes, metabolites and accumulation of metals are responsible for reduction of metal ions and helping in the formation nanoparticles. Raja et al. 2021 reported that biologically synthesized nanoparticles by using cell free culture filtrate of *T. harzianum* Th3 inhibits the mycelial growth of groundnut root rot complex pathogens by 60–65%. Production of secondary metabolites during mycoparasitism also a pivotal key of *Trichoderma* spp. in the antagonistic mechanism. For example, secondary metabolites like harzianic acid (HA), 6-pentyl-α-pyrone (6PP), koninginin, harzianopyridone and etc. can be correlated with biocontrol mechanisms (Vinale and Sivasithamparam 2020).

Plant growth promoting fungi (PGPF) are majorly associated with wide range of hosts and helps in transformation of soil nutrients, alter the niche of rhizosphere, elucidate the systemic resistance, and improve the plant growth. *Trichoderma* spp. are one of major beneficial fungal community present in the soil environment which directly create an impact on plants such as increased in number lateral roots and length, cumulative root length and root tips, germination efficacy and seeding growth, improved surface area of roots and leaves, wet and dry weight of plant biomass, and positive effect on flowering. And also responsible for elucidation of plant immunity through increasing jasmonic acid (JA), salicylic acid (SA), ethylene (ET), phytoalexin levels and root exudates in plants, soil nutrients solubilization, and nutrient uptake. Some of the *Trichoderma* spp. are rhizospheric competent in nature that can be able to colonize the plant

roots and enter the epidermis and outer cortex of root system (Harman et al. 2004). Recently Nofal et al. (2021) reported that seedling treatment with 10% cell free culture filtrate of *T. atroviride* from rhizosphere of tomato could improve the plant growth and decreased wilt incidence percentage (8%) caused by *Fusarium oxysporum* f. sp. *lycopersici*. The current study also revealed the impact of seed inoculation with selected native *Trichoderma* isolates which helps in improvement of germination efficacy, root, and shoot length in groundnut. The RWC of the root and shoot in treated plants has been increased which indicates the acceleration in the plant growth. Based on the obtained results, the highly efficient strain *T. harzianum* Thar23 exhibits excellent mycelial growth inhibition of pathogen, lytic enzymes production and improve the plant growth could be used against biotic and abiotic stress at greenhouse and field level in pest management practices.

Performance of microbial antagonistic under field condition is one of the important key factors in commercialization of the product at market level. The present findings were in accordance with several research findings stated that the importance of performance of *Trichoderma* spp. against reduction of different pathogen population at field level (Sharma et al. 2012; Jambhulkar et al. 2022). In this present study, there are differences in performance of *Trichoderma* isolates, however treatment with *T. harzianum* Thar23 enhanced groundnut growth, reduction in *S. rolfsii* disease incidence, significant increase in shelling percentage and pod yield among other isolates.

Conclusion

Based on morpho and molecular characterization 35 native *Trichoderma* isolates were grouped into four different *Trichoderma* spp. namely, *T. asperellum* (12), *T. harzianum* (11), *T. Longibrachiatum* (6) and *T. citrinoviride* (6) from rhizosphere of groundnut and screened based on the antagonistic activity against *S. rolfsii.* The potential isolates from each group viz*., T. harzianum* Thar23, *T. asperellum* Tasp49, *T. longibrachiatum* Tlongi5 and *T. citrinoviride* Tcitri2 were selected for lytic enzyme production and plant growth promoting studies in groundnut. The highly efficient isolate *T. harzianum* Thar23 exhibits excellent mycelial growth inhibition of pathogen, lytic enzymes production and improves the plant growth which could be used in biotic and abiotic stress management in groundnut at both green house and field level.

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Declarations

Conflict of interest The authors declare no competing of interest.

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