Template/Pro forma for Submission

NMHS-Himalayan Institutional Project Grant

NMHS-FINAL TECHNICAL REPORT (FTR)

Demand-Driven Action Research and Demonstrations

NMHS Grant Ref. No.:	GBPNI/NMHS-2018-19/MG 1

Date of Submission:	0	5	0	7	2	0	2	3
	d	**d**	**m**	**m**	**y**	**y**	**y**	**y**

PROJECT TITLE (IN CAPITAL)

DEVELOPMENT OF MICROBIAL INOCULANTS TO IMPROVE GROWTH AND PRODUCTIVITY OF DARJEELING AND ASSAM TEA

Project Duration: *from* (**21.01.2019**) *to* (**31.10.2022**).

***Submitted to*:**
Er. Kireet Kumar
Scientist 'G' and Nodal Officer, NMHS-PMU
National Mission on Himalayan Studies, GBP NIHE HQs
Ministry of Environment, Forest & Climate Change (MoEF&CC), New Delhi
E-mail: nmhspmu2016@gmail.com; kireet@gbpihed.nic.in; kodali.rk@gov.in

***Submitted by*:**
[*R. K Chauhan*]
[*DTRDC, Tea Board, Kurseong*]
[*Contact No.9475522006*]
[*E-mail:rjshchauhan3@gmail.com*]

GENERAL INSTRUCTIONS:

1. The Final Technical Report (FTR) has to commence from the start date of the Project (as mentioned in the Sanction Order issued by NMHS-PMU) till completion of the project duration. Each detail has to comply with the NMHS Sanction Order.
2. The FTR should be neatly typed (in Arial with font size 11 with 1.5 spacing between the lines) with all details as per the enclosed format for direct reproduction by photo-offset printing. Colored Photographs (high resolution photographs), tables and graphs should be accommodated within the report or annexed with captions. Sketches and diagrammatic illustrations may also be given detailing about the step-by-step methodology adopted for technology development/ transfer and/ or dissemination. Any correction or rewriting should be avoided. Please provide all information under each head in serial order.
3. Any supporting materials like Training/ Capacity Building Manuals (with detailed contents about training programme, technical details and techniques involved) or any such display material related to project activities along with slides, charts, photographs should be brought at the venue of the Annual Monitoring & Evaluation (M&E) Workshop and submitted to the NMHS-PMU, GBP NIHE HQs, Kosi-Katarmal, Almora 263643, Uttarakhand. In all Knowledge Products, the Grant/ Fund support of the NMHS should be duly acknowledged.
4. The FTR Format is in sync with many other essential requirements and norms desired by the Govt. of India time-to-time, so each section of the NMHS-FTR needs to be duly filled by the proponent and verified by the Head of the Lead Implementing Organization/ Institution/ University.
5. Five (5) hard-bound copies of the Project Final Technical Report (FTR) and a soft copy of the same should be submitted to the **Nodal Officer, NMHS-PMU, GBP NIHE HQs, Kosi-Katarmal, Almora, Uttarakhand**.

The FTR is to be submitted into following two (02) parts:

Part A – Project Summary Report

Part B –Detailed Project Report

In addition, the Financial and other necessary documents/certificates need to be submitted along with the Final Technical Report (FTR) as follows:

Annexure I	**Consolidated and Audited Utilization Certificate (UC) & Statement of Expenditure (SE)**, including the interest earned for the last Fiscal year and the duly filled GFR-19A (with year-wise break-up).
Annexure II	**Consolidated Interest Earned Certificate**
Annexure III	**Consolidated Assets Certificate** showing the cost of the equipment in Foreign/ Indian currency, Date of Purchase, etc. (with break-up as per the NMHS Sanction Order and year wise).
Annexure IV	**List of all the equipment, assets and peripherals** purchased through the NMHS grant with current status of use, including location of deployment.
Annexure V	**Transfer of Equipment** through Letter of Head of Institution/Department confirming the final status of equipment purchased under the Project.
Annexure VI	**Details, Declaration and Refund of any Unspent Balance transferred through Real-Time Gross System (RTGS)/ PFMS in favor of NMHS GIA General**

NMHS-Final Technical Report (FTR) *template*

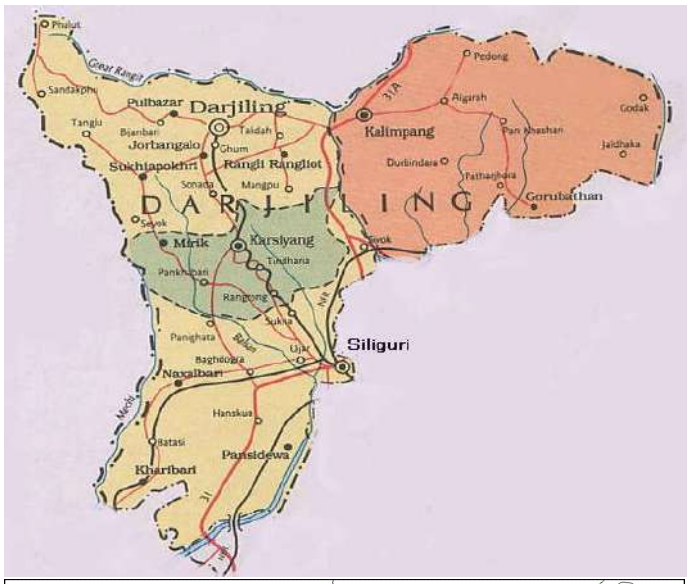
Demand-Driven Action Research Project

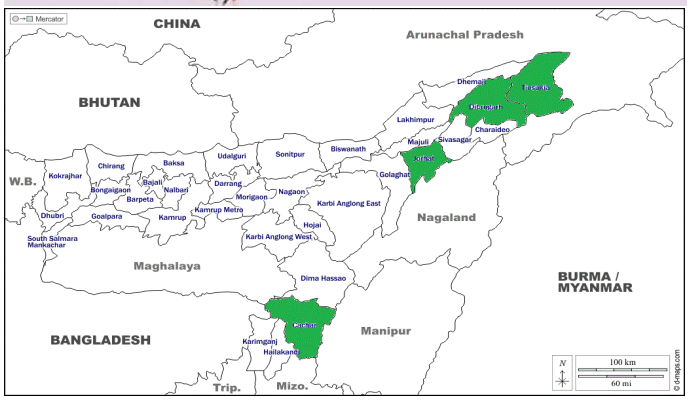
DSL: Date of Sanction Letter	*DPC: Date of Project Completion*
21012019	31102022
ddmmyyyy	ddmmyyyy

Part A: Project Summary Report

1. Project Description

i.	Project Grant Ref. No.:	GBPNI/NMHS-2018-19/MG 1 Dated:21.01.2019
ii.	Project Category:	Small Grant [ ] Medium Grant [√] Large Grant [ ]
iii.	Project Title:	Development of Microbial Inoculants to Improve Growth and Productivity of Darjeeling and Assam Tea
iv.	Project Sites (IHR States/ UTs covered) (*Location Maps attached*):	Darjeeling, West Bengal and Assam IHR State covered: West Bengal and Darjeeling Long. & Lat.: Darjeeling 27.0410° N, 88.2663° E Assam 26.2006° N, 929376° E





v.	Scale of Project Operation:	Local		Regional		Pan-Himalayan	
vi.	Total Budget:	0.736 (in Cr)					
vii.	Lead Agency:	Tea Board of India					
	Lead PI/ Proponent:	Dr Biswajit Bera					
	Co-PI/ Proponent:	Rajesh Kumar Chauhan					
viii.	Implementing Partners:	DTRDC, Tea Board, Darjeeling Assam University, Assam National Centre for Cell Science, Pune, Maharashtra					
	Key Persons (Contact Details, Ph. No., E-mail):	**DTRDC, Tea Board** Rajesh Kumar Chauhan Contact:9475522006 Email: rjshchauhan3@gmail.com Dr. Biswajit Bera Ex. Director Research Contact:9836844111 Email: dr1teaboard@gmail.com **Assam University** Prof. Piyush Pandey Contact:9476600630 Email: piyushddn@gmail.com **National Centre for Cell Science** Dr. Praveen Rahi Contact:9836844111 Email: Praveen_rahi22@yahoo.co.in					

2. Project Outcomes

2.1. Abstract/ Summary (not more than 250-300 words)

Background: Tea (*Camellia sinensis*) is the world's most popular and economic beverage due to its distinctive fragrance and flavor generated by the leaves of commercially cultivated tea plants. The tea microbiome has now become a prominent topic of attention for microbiologists in recent years as it can help the plant for soil nutrient acquisition as well as stress management. The plants and rhizospheric soil are the hotspots of native microbes playing critical roles in nutrient recycling and maintenance of plant ecosystems. The several groups of microbes are the key components of soil–plant systems, where they are engaged in a concentrated link of rhizosphere and endophyte interactions in plant microbiome.

Objectives: The aim of the present study is to collect, isolate and screen out the microbes and their diversity associated with plant microbiome. Plant growth promoting microbes stimulate plant growth directly or indirectly by releasing plant growth regulators, solubilization of phosphorus, potassium, and zinc, biological nitrogen fixation and by producing secondary metabolites i.e. IAA, ACC. The evaluation of isolates in in-situ and ex-situ condition under indigenous ecosystem. The demonstration cum hands-on training conducted to technology transfer to stakeholders.

Methodology: Tea rhizosphere soil samples were collected from different tea estates of Assam and Darjeeling, India in a sterile zip-lock plastic bags and stored at 4^0C. Isolation and characterization of bacterial isolates were conducted by serial dilution method. Screening for PGP traits were performed and the isolates showing best result for PGP traits were selected and consortium were prepared and applied on the Pot trial and Field trial. Field experiment was conducted at Darjeeling and Assam with the consortium developed under the project with *Camellia sinensis* plant for evaluation of their effect on chemical and organic inputs.

Result: The yield attribute was increased with the application of consortium with higher doses of organic and inorganic inputs in one plucking year of tea. Tea quality such as total polyphenol, TF, and TR content were evaluated to determine the effect of consortium along with fertilizer and manure in made tea. The plant enzymes Superoxide dismutase (SOD), Total phenolic content (TPC), Catalase (CAT) and Glutathione reductase (GSH) activity along with the total Chlorophyll content were assessed to determine the effect of consortium and fertilizer in plant growth. The activity of enzymes GSH, TPC, SOD, CAT and the total chlorophyll content were increasing in each growing condition. Increase in the activity of these enzymes and the chlorophyll level depicts improved plant health. Also, increased level of chemical toxicity in plant were nullified with the effect of consortium. The effect of consortium on soil physical properties was also assessed and increasing content of nitrogen phosphorus and potassium in soil and leaf shoot revealed that the use of bio-inoculant in field sustained the productivity and production in ecofriendly system.

Conclusion: Though the work has concentrated on microbiome erection and its function under natural indigenous environment. This research may enable to manage the microbes for increased production and productivity in hilly region of Darjeeling and Assam. Thus, the microbes isolated through this research may be further applied on the tea gardens as a bio-inoculants to the increase plant health and suppressed the residual effect of chemical fertilizer.

Recommendations: The microbes associated with plant growth-promoting qualities have developed from indigenous species of hilly region as an important and promising tool for sustainable agriculture.

2.2. Objective-wise Major Achievements

S#	Objectives	Major achievements (***in bullets points***)
1	Deciphering the microbial communities associated with tea cultivated in Darjeeling and Assam regions of India	• A total of 146 microbes have been isolated and 26 from Darjeeling and 8 strains from Assam were selected after screening all the PGPR traits for further analysis. The isolates that have showed positive results in all the PGPR test conducted like (Phosphate solubilization, Siderophore, IAA and ACC deaminase) were selected for further analysis and experiments. The results of the isolates that was recorded positive for PGPR traits. • In Darjeeling and Assam, several microorganisms have been identified including *Bacillus cereu, Yokenella regensburgei, Lelliottia amnigena, Bacillus vireti, Bacillus megaterium, Lactobacillus coleohominis, Bacillus safenesis, Bacillus licheniformis, Bacillus badius, Bacillus pumilus, Bacillus firmus, Bacillus mycoides, Lysinibacillus fusiformis, Lysinibacillus fusiformis, Serratia marcescens, Klebsiella aerogenes* and *Bacillus thuringiensis*. • *Bacillus cereus* is known for its ability to produce antimicrobial compounds that can inhibit the growth of pathogenic bacteria. *Yokenella regensburgei*, on the other hand, has been found to have plant growth-promoting properties, enhancing the overall health and productivity of plants. *Lelliottia amnigena* is known for its nitrogen-fixing abilities, which can contribute to the fertility of soil in the region. • Similarly, *Bacillus vireti*, *Bacillus megaterium*, *Lactobacillus coleohominis*, *Bacillus safenesis*, *Bacillus licheniformis*, *Bacillus badius*, *Bacillus pumilus*, *Bacillus firmus*, *Bacillus mycoides*, and *Lysinibacillus fusiformis* have all shown different beneficial traits. These include phosphate solubilization, production of plant growth-promoting hormones, and suppression of plant pathogens. Lysinibacillus fusiformis, which is also found in Darjeeling and Assam both, has been found to exhibit similar plant growth-promoting properties. Serratia *marcescens*, on the other hand, is known for its ability to produce various enzymes that can degrade organic matter in the soil. • The entophytic and rhizospheric microbes isolated from Darjeeling and Assam exhibit a wide range of beneficial traits. These microbes have the potential to enhance plant growth, improve soil fertility, and suppress the growth of harmful pathogens.

2	To determine the effect of chemical inputs on yield in correlation to change in microbiome structure, and its comparative assessment in different zones	• Based on the findings from the field trials conducted in both Darjeeling and Assam, it is evident that the combination of chemical fertilizer, organic manure, and bio-inoculants is a promising approach to achieve higher crop productivity and improved quality attributes. The incorporation of bio-inoculants helps in enhancing soil fertility and suppressing diseases, while organic manure contributes to the overall nutrient availability. These findings can be utilized by farmers and agricultural practitioners to optimize their farming practices and achieve sustainable agricultural production. • The assessment of quality and yield attributes revealed interesting findings. In Darjeeling and Assam, the combination of chemical fertilizer, organic manure, and bio-inoculants exhibited the highest crop yield across various clones. This treatment not only improved yield but also enhanced the quality attributes such as polyphenol, theaflavin, and thearubigin composition. The bio-inoculants played a crucial role in enhancing soil fertility and promoting beneficial microbial activity, resulting in improved overall crop performance. • The consortium treated plot exhibited a noteworthy boost in crop yield, quality, and nutrient absorption compared to the control group. This significant improvement can be attributed to the enhanced activity of Plant Growth Promoting Rhizobacteria (PGPR) under the natural conditions of the indigenous microbial community. The utilization of the consortium treatment led to a remarkable increase in the overall productivity of the tea, as well as the nutritional value of the made tea. These findings highlight the potential benefits of utilizing PGPR in agricultural practices, particularly in terms of optimizing plant growth and maximizing nutrient uptake. • The combination of bio-inoculants of indigenous origin and antimicrobial exudates produced by PGPR offers a promising solution to mitigate the residual effects of chemical inputs in field experiments. By harnessing the power of beneficial microorganisms and their interactions with plants, farmers can improve crop productivity while minimizing the negative environmental impact associated with conventional agricultural practices.
3	To isolate the key microbial species using improved cultivation techniques and identification using MALDI-TOF MS and DNA sequencing	• MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) and 16S rRNA gene sequencing are two commonly used methodologies in microbiology for microbial identification and classification under the project.

4	Screening of microbial strains for multiple plant growth promoting attributes	• Characterizing the plant growth-promoting properties of bacterial isolates to understanding their potential in sustainable tea cultivation. This study demonstrated the diverse range of traits exhibited by the isolates, including hormone production, phosphate solubilization, nitrogen fixation, induction of systemic resistance, and production of hydrolytic enzymes. Further the following parameters have explored for specific mechanism underlying plant microbe interaction: i. Phosphate Solubilizing test ii. Siderophore Producing test iii. Indole Acetic Acid (IAA) test iv. ACC deaminase assay v. Potassium solubilization vi. Zinc Solubilization vii. Hydrogen cyanide (HCN) production viii. Ammonia production ix. Protease Activity
5	Development of microbial formulation based on efficient strains and field demonstration in tea gardens	• To assess the effectiveness of the microbial formulation, field experiments are conducted in tea gardens and comparing the results with conventional practices. The collected data from field experiment have statistically analysed to determine the significance of any observed differences between the microbial formulation and conventional practices. This analysis helps establish the effectiveness of the microbial formulation in tea gardens. • The development of a microbial formulation based on efficient strains and its subsequent field demonstration in tea gardens provide promising avenues for sustainable tea cultivation practices. By harnessing the power of beneficial microorganisms, tea growers can reduce their reliance on chemical inputs, promote environmental stewardship, and enhance tea quality.

6	Impart training to tea workers to produce and apply the bio-inoculant	• The hands-on training programme organized for multiplication and field application in the tea field for small tea growers of Darjeeling and Assam were a valuable opportunity for participants to enhance their skills and knowledge. The practical training and field application exercises provided them with the necessary tools and techniques to improve their tea cultivation practices. This programme was undoubtedly a milestone to contributed to the overall growth and development of the tea industry in the region. • The distribution of the developed consortium among the participants for further development of microbial enriched compost/vermicompost at their own fields was a strategic and effective approach. It not only encouraged collaboration and knowledge sharing, but also empowered the participants to actively contribute to the advancement of composting practices and ultimately improve the quality and productivity of their farming endeavors. • Microbial enriched compost is a valuable resource for sustainable agriculture. By following the methodology outlined in this training manual, farmers and gardeners can prepare high-quality compost that enhances soil fertility, promotes plant growth, and improves overall soil health. The proper rate of application for further compost preparation and field application ensures optimal results and long-term benefits. • The feedback of growers is crucial in evaluating the effect of using microbial inoculants enriched with organic material in the field. By collecting and analyzing this feedback, we can gain valuable insights into the effectiveness of this practice, identify areas for improvement, and continue to enhance the use of microbial inoculants for sustainable agriculture.

Note: Further details may be summarized in DPR Part-B, Section-5. Supporting materials may be enclosed as annexure/ appendix separately to the FTR.

2.3. Outputs in terms of Quantifiable Deliverables*

S#	Quantifiable Deliverables*	Monitoring Indicators*	Quantified Output/ Outcome achieved	Deviations, if any, & Remarks thereof:
1	Details database of the population	Number of key microbial species/	Several microbes have isolated out of	

	dynamics of bacteria and their functional role, associated with tea plant in Darjeeling and Assam.	strain isolated (Nos.)	them 29 from Darjeeling and 8 from Assam was observed prominent.	
2	A Collection of microbial strains with plant growth promoting (PGP) activity	Developed Demonstration model (Nos.)	The pot trial and field trial have generated a good result in plant growth parameters and its enzyme activity.	
3	Establishing field demonstrations at 2 farms in selected sites	Database developed (Nos.)	Microbial data was developed	
4	Improvement in / income of tea worker by least 30-40% through crop improvement	Improved the economic condition/income (%)	Small tea growers producing biofertilizer enriched compost and maximizing their yield	
5	Imparting training to at least 100 SC/ST and other tea workers in 2 sites	Number of Training Programme Organized (Nos.)	Three hands-on training programme was organized at different places Darjeeling hills	
6		Number of beneficiaries village/ local people (Nos.)	Total 80 small tea growers were participated in training programme	
		No. of Reports/Research articles/Policy documents prepared and published (Nos.)	Nil	

*As stated in the Sanction Letter issued by the NMHS-PMU.

2.4. **Strategic Steps with respect to Outcomes** (in bullets)

S#	Particulars	Number/ Brief Details	Remarks/ Attachment
1.	New Methodology/ Technology developed, *if any*:	Nil	
2.	New Ground Models/ Process/ Strategy developed, *if any*:	Nil	
3.	New Species identified, *if any*:	29 from Darjeeling and 8 from Assam region	
4.	New Database established, *if any:*		

S#	Particulars	Number/ Brief Details	Remarks/ Attachment
5.	New Patent, *if any*:	Nil	
	I. Filed (Indian/ International)	Nil	
	II. Technology Transfer, *if any*:	Nil	
6.	Others, *if any*	Nil	

Note: Further details may be summarized in DPR Part-B, Section-5. Supporting materials may be enclosed as annexure/ appendix separately to the FTR.

3. New Data Generated over the Baseline Data

S#	New Data Details	Status of Existing Baseline	Addition and Utilisation New data
1		**Microbial database of Darjeeling and Assam has been developed**	**Developed inoculants may be utilized for sustainable production with quality.**

Note: Further details may be summarized in DPR Part-B. Database files in the requisite formats (Excel) may be enclosed as annexure/ appendix separately to the soft copy of FTR.

4. Demonstrative Skill Development and Capacity Building/ Manpower Trained

S#	Type of Activities	Details with number	Activity Intended for	Participants/Trained			
				SC	**ST**	**Women**	**Total**
1.	Workshops						
2.	On-Field Trainings	**4**	**Distribution of inoculum in the form of consortium, developed from indigenous soil of tea**				**100**
3.	Skill Development						
4.	Academic Supports	**Nil**					
	Others (if any)	**Nil**					

Note: Further details may be summarized in DPR Part-B. Supporting materials may be enclosed as annexure/ appendix separately to the FTR.

5. Linkages with Regional & National Priorities (SDGs, INDC, etc.)/ Collaborations

S#	Linkages /collaborations	Detail of activities (No. of Events Held)*	No. of Beneficiaries

1.	Sustainable Development Goals (SDGs)/ Climate Change/INDC targets addressed	Sustainable production has been successfully achieved through the use of a consortium approach combined with organic and inorganic cultivation practices. This collaborative and environmentally friendly approach not only minimizes the negative impacts of production on the environment but also enhances the social and economic well-being of stakeholders. By adopting sustainable production methods, companies can meet consumer demand for environmentally friendly products and contribute to a more sustainable future.	100
2.	Any other:		

Note: Further details may be summarized in DPR Part-B, Section-6. Supporting materials may be enclosed as annexure/ appendix separately to the FTR.

6. Project Stakeholders/ Beneficiaries and Impacts

S#	Stakeholders	Support Activities	Impacts in terms of income generated/green skills built
1.	Line Agencies/ Gram Panchayats:		
2.	Govt Departments (Agriculture/ Forest/ Water):		
3.	Villagers/ Farmers:	In order to enhance their knowledge and expertise in multiplication and field application in the tea field, a training programme was organized in Darjeeling and Assam.	The training programme focused on various aspects of multiplication and field application in the tea field. Participants were taught about the techniques and methods involved in the multiplication and application of bio-inoculum at their own field.
4.	SC Community:		
5.	ST Community:		
6.	Women Group:		
	Others, *if any*:		

Note: Further details may be summarized in DPR Part-B, Section-6. Supporting materials may be enclosed as annexure/ appendix separately to the FTR.

7. Financial Summary (Cumulative)

Please attach the consolidated and audited Utilization Certificate (UC) and Year-wise Statement of Expenditure (SE) separately, *ref*. **Annexure I.**

8. Major Equipment/ Peripherals Procured under the Project** (*if any*)

S#	Name of Equipment	Quantity	Cost (INR)	Utilisation of the Equipment after project
1.	Spectrophotometer	1	2.50Lakh	Used for estimation of nutrient content in leaves and soil.
2.	Oxford Nanopore sequencer with accessories	1	6.00Lakh	To determine the sequence of DNA/RNA bases.
3	High-end workstation	1	4.00Lakh	

Details should be provided in details **(*ref.* **Annexure III &IV).**

9. Quantification of Overall Project Progress

S. No.	Parameters	Total (Numeric)	Remarks/ Attachments/ Soft copies of documents
1.	IHR States/ UTs covered:	2	
2.	Project Sites/ Field Stations Developed:	2	
3.	Scientific Manpower Developed (PhD/M.Sc./JRF/SRF/ RA):	3	
4.	Livelihood Options promoted	100	
5.	Technical/ Training Manuals prepared	2	
6.	Processing Units established, if any		
7.	No. of Species Collected, if any	146	
8.	No. of New Species identified, if any		
9.	New Database generated (Types):		
	Others (if any)		

Note: Further details may be summarized in DPR Part-B. Supporting materials may be enclosed as annexure/ appendix separately to the FTR.

11. Knowledge Products and Publications:

S#	Publication/ Knowledge Products	*Number*		*Total Impact Factor*	*Remarks/* Enclosures
		National	International		
1.	Journal – Research Articles/ Special	Nil	Nil		

S#	Publication/ Knowledge Products	*Number*		*Total Impact Factor*	*Remarks/* Enclosures
		National	International		
	Issue:				
2.	Book – Chapter(s)/ Monograph/ Contributed:	Nil	Nil		
3.	Technical Reports:	Nil	Nil		
4.	Training Manual (Skill Development/ Capacity Building):	Anexure	Nil		
5.	Papers presented in Conferences/Seminars:	Nil	Nil		
6.	Policy Drafts/Papers:	Nil	Nil		
7.	Others, if any:	Nil	Nil		

Note: Please append the list of KPs/ publications (with impact factor, DOI, and further details) with due Acknowledgement to NMHS. Supporting materials may be enclosed as annexure/ appendix separately to the FTR.

12. Recommendation on Utility of Project Findings, Replicability and Exit Strategy

Particulars	Recommendations
Utility of the Project Findings:	• Enhancing the microbiome association with the tea plant can result in several benefits. Firstly, it can improve nutrient uptake and utilization by the tea plant, leading to healthier and more vigorous growth. This, in turn, can enhance the overall yield and quality of tea produced. • Moreover, a well-balanced and diverse microbiome can contribute to the plant's defense against pathogens and pests. By promoting a beneficial microbial community, the tea plant becomes more resilient and less susceptible to diseases and other stressors. This reduces the reliance on chemical inputs, thereby fostering sustainable and environmentally friendly tea production practices. • In addition to its impact on plant health, the improved microbiome association can also have positive effects on soil fertility and ecosystem sustainability. Microorganisms play a crucial role in soil nutrient cycling and organic matter decomposition. A thriving microbiome can enhance soil structure, moisture retention, and nutrient availability, thus benefiting the tea plant and the entire ecosystem.

	• The utility of these project findings extends beyond the tea industry. The knowledge gained from studying the microbiome association with the tea plant can be applied to other crops as well. It provides a framework for understanding the significance of microbial interactions in promoting plant growth and can guide future research in sustainable agriculture. • The project findings on isolates that enhance the microbiome association with the tea plant offer valuable insights and tools for sustainable tea production. By harnessing the power of beneficial microbial communities, tea growers can improve plant health, increase yield, reduce reliance on chemicals, and contribute to a more sustainable and resilient agricultural system. These findings have broader implications for sustainable agriculture as a whole, highlighting the importance of understanding and harnessing the potential of the plant-microbiome relationship.
Replicability of Project/ Way Forward:	• The development of microbial inoculants to enhance the growth and productivity of Darjeeling and Assam tea holds immense potential for sustainable tea production. The replicability of this project is vital for its successful implementation on a larger scale. By documenting the development process, creating standard procedure, and promoting knowledge transfer, this project aims to enable tea growers to replicate and benefit from the use of microbial inoculants, ultimately improving the quality and quantity of tea produced in these regions.
Exit Strategy:	• One of the key elements of the exit strategy is to establish self-sustainability within the consortium. This involves equipping small tea growers with the necessary knowledge, skills, and resources to independently manage their operations and navigate the tea industry. Through training programs and capacity-building initiatives, the consortium aims to empower growers to make informed decisions and adapt to changing market dynamics. By facilitating their transition from dependence on external support to self-sufficiency will ensures that the consortium becomes a self-sustaining entity.

	• The project work also emphasizes the importance of benefit to the stakeholders involved in the consortium multiplication and application themselves. By implementing organic practices, the consortium aims to increase the yield and soil health of the tea plantations. This not only enhances the quality of the tea produced but also contributes to the financial stability of the growers. • The project aims to create a ripple effect by generating socio-economic opportunities for the community surrounding the tea plantations. This may include job creation, skill development, and ecosphere management initiatives. By involving the local community and ensuring their active participation, it was seeking to build a sustainable ecosystem that benefits not only the growers but also the broader community. • In conclusion, the consortium develop under the project for small tea growers is designed to achieve self-sustainability and maximize the benefits for stakeholders and the local community. By empowering growers to independently manage their operations, adopting organic practices to enhance yield and soil health, and creating socio-economic opportunities for the community and ensures the long-term growth and financial stability of the tea industry.

(**PROJECT PROPONENT/ COORDINATOR**)

(Signed and Stamped)

(**HEAD OF THE INSTITUTION**)

(Signed and Stamped)

Place: ……..
Date: …../……/……

PART B: DETAILED PROJECT REPORT

The Detailed report should include an Executive Summary and it should have separate chapters on (i) **Introduction**, (ii) **Methodologies/Strategy/Approach**, (iii) **Key Findings and Results**, (iv) **Overall Achievements**, (v) **Project's Impacts in IHR** (vi) **Exit Strategy** and Sustainability, (vii) **References**, and (viii) **Acknowledgements** (acknowledging the financial grant from the NMHS, MoEF&CC, GoI).

Other necessary details/ Supporting Documents/ Dissemination Materials (*New Products/ Manuals/ Standard Operating Procedures (SOPs)/ Technology developed/Transferred, etc, if any*) may be attached as Appendix(ces).

1 EXECUTIVE SUMMARY (not more than 2–3 pages)

Tea rhizosphere soil samples were collected from different tea estates of Assam and Darjeeling, India in a sterile zip-lock plastic bags and stored at 4^0C. Isolation and characterization of bacterial isolates were conducted. Screening for PGP traits were performed and the isolates showing best result for PGP traits were selected and Consortium were prepared and applied on the Pot trial and Field trial. Pot trial and field trial experiments have been set up with *Camellia sinensis* plant with five different growing conditions – (a) control (only plant), (b) bacterial consortium, (c) 25% fertilizer + consortium, (d) 50% fertilizer + consortium, and (c) Fertilizer(only). The Pot trial was for a period of Six months and the Field trial was for a period of one year. The plant enzymes Superoxide dismutase (SOD), Total phenolic content (TPC), Catalase (CAT) and Glutathione reductase (GSH) activity along with the total Chlorophyll content were assessed to determine the effect of consortium and fertilizer in plant growth. The activity of enzymes GSH, TPC, SOD, CAT and the total chlorophyll content were increasing in each growing condition. Increase in the activity of these enzymes and the chlorophyll level depicts improved plant health. Also, increased level of chemical toxicity in plant were nullified with the effect of consortium.

2 INTRODUCTION

2.1 Background (max. 500 words)

Tea (*Camellia sinensis*) is the world's most popular and economic beverage due to its distinctive fragrance and flavour generated by the leaves of commercially cultivated tea plants. The tea microbiome has now become a prominent topic of attention for microbiologists in recent years as it can help the plant for soil nutrient acquisition as well as stress management. Tea is among the most significant industrial crops, as well as the most extensively consumed non-alcoholic beverages throughout the world (Bora and Bora, 2021). Despite an upsurge in growing land, Indian tea output and exports have exhibited a decreasing tendency in recent times. Pathogens and diseases are two of the most significant problems impeding productivity. Infections in tea frequently result in inferior beverage quality and crop damage, resulting in significant financial losses to the sector. Tea plants are perennials and are mostly grown in a warm humid climate. For the control of weeds and tea pest, chemicals are widely used in tea fields. Inappropriate usage of synthetic chemicals to control pests and illnesses has also become a major problem for the tea business.

Most of the tea growing area is in progress towards organic farming. The tea plants are growing in same soil from more than 100 years through conventional farming. Fertilizer application to explore the high production and increased quality concept is getting down day by day due to its environmental consequences and residual content in made tea. Organic farming without using any pesticide, fertilizer may be a sustainable and eco-friendly approach for better & healthy production.

The microclimate of tea bushes is depending on the density of plant, morphology of leaves, slope gradient and altitude of plantation. The aerial part of plant is denoted as phyllosphere while below ground the part is referred as rhizosphere. The plant microbiome consists of several microbial communities like bacteria, fungi algae and protozoa, which played important roles in beneficial or harmful effect to the ecosystem. Rhizospheric soil is anchorage of several microbes, which may involve in nutrient cycling, regulating soil fertility, maintaining plant health and productivity.

The rhizospheric soil of hilly region contains various types of PGPR community. Numerous research studies are conducted on the understand of the diversity, dynamics and importance of soil PGPR in agricultural productivity. PGPR associated with tea rhizosphere is reported previously (Jintu Dutta & Debojit Thakur 2017). Some common PGPR examples are Azospirillum, Azotobacter, Bacillus, Burkholdaria, Enterobacter and Rhizobium etc are uses in different agricultural crops. The growth promoting resulting nitrogen fixation, phosphate mobilization, exudation of secondary metabolites such as auxin, cytokinin, indole acitic acid etc. PGPR can be used as an element in organic management systems in which the residual effect of agrochemicals may be controlled.

2.2 Overview of the major issues addressed (max. 500 words)

Unlike other Indian teas, mostly obtained from Assam tea clones (*Camellia sinensis* var. *assamica*), Darjeeling tea is obtained from the small leaved Chinese tea bush, *C. sinensis* var. *sinensis* have its greater impact on world tea market due to their superiority in flavour and taste. The excellence and physiognomies of Darjeeling tea are fundamentally attributable to its geographic derivation. Tea microbes remained a prominent research topic for scientists over the years as tea microbes helps in nutrient cycling and stress management which in turn improve the tea growth, yield and quality. The roots of tea plants are colonized by various microbes including arbuscular mycorrhizal fungi (AMF), bacterial communities, and endophytes increase root growth, development and nutrient uptake which in turn improve tea growth, yield and quality. These microbes also increase the concentration of nutrients, amino acids, soluble proteins, flavonoids, catechuic acid, glucose, fructose, sucrose contents caffeine, and polyphenols concentration in tea plants. Besides this, microbes also protect the tea plants from harmful pest and diseases which in turn leads to an appreciable improvement in plant growth and development. The most important goal of any farming system is to establish a system with production of maximum food

while minimizing impacts on the environment. The present focused on the role of various microbes in improving the growth, yield and quality of tea plants.

The microorganisms play a pivotal role in nutrient cycling, regulating soil fertility, maintaining plant health, and productivity. It is key factor to study microbial diversity to understand the relationship between rhizosphere, phylosphere and atmosphere. Furthermost of the microorganisms mainly employ plant root-derived nutrients such as root exudates in the vicinity of root i.e. rhizosphere. It is assessed that each gram of soil comprehends more than lakhs of different species. The useful rhizospheric microbes are usually mentioned to as plant growth-promoting rhizobacteria which is beneficial for plant growth and soil health. The secretion of root exudates in rhizosphere enhances the physical and chemical condition of soil and consequently stimulate the microbial diversity. The dynamics of soil microbes have important inferences for the reaction of subsurface soil ecosystems. Soil microbial diversity are representing the variability of organisms such as bacteria, archaea, fungi, algae, and viruses.

Tea plants are perennials and are mostly grown in a warm humid climate. For the control of weeds and tea pest, chemicals are widely used in tea fields. In the consequences of extreme use of chemicals such as fertilizers, fungicides and pesticides in tea fields in long period of time declined the tea production and deteriorate the soil fertility. Hence, there is a need for eco-friendly approach to reduce the use of chemical application. To overcome this situation, the plant growth promoting rhizobacteria will play a key role to enhance plant health and productivity and quality without environmental pollution.

The PGPR associated with tea rhizosphere in tea growing area with the aim of sustainable tea production is focused. In this study, the molecular techniques have been applied to investigate the genetic diversity of tea PGPR. PCR based genotype method such as amplification with a universal 16S primer set consisting of 27F and 1492R and MALDI-TOF MS was used to evaluate microbial microbial diversity in wide environmental sites of Darjeeling and Assam.

2.3 Baseline Data and Project Scope (max. 500 words)

Assam and Darjeeling tea is very famous in the world and having a good position in tea industry. Geographical location and climate of Darjeeling and Assam are favorable for its unique tea production. Tea crop grows in these two region having several biotic and abiotic stress like pH, water scarcity, weed and insect pest management which lead to the considerable loss of crop every year. To overcompensate the problems raised from indiscriminate use of chemical is an alternative approach is the necessity of present work. Thus, this research was carried out to explore the possibilities of PGPR population present in the tea rhizosphere soil which could be used in the sustainable tea cultivation. Total 146 inoculums were isolated from different tea estates of Darjeeling and Assam. Furthermore, 38 prominent microbes were screened out for further PGPR study. The ultimate aim of this work is to create an effective bio-inoculants for tea growing region of Assam and Darjeeling. In future the exploitation and production of indigenous bio-inoculant based on tea microbiome and their potential use in tea cultivation may enable us to manipulate tea microbiome to develop management strategies for increased production and

productivity. The leaving microorganism are capable to exudates PGPR and colonize in rhizospheric zones are stimulate the availability of nutrients resulting growth of yield attributes along with suppression of harmful impact of agrochemicals and pesticide. The mechanism of PGPR to stimulate the growth is directly by solubilization of nutrients and indirectly by the induced biochemical changes which are activated by PGPR promote resistance of plant against specific disease and pest.

In the changed scenario of climate resulting heavy rainfall in short period, heavy cold and increasing temperature and draught affected the sustainable production and productivity of tea. Under such condition an environment friendly mechanism is necessity to overcome the such situation and indiscriminate use of agrochemicals in the field. The systematic application of plant growth promoting rhizobacteria in soil will be an alternative source of balance nutrition to plants which improves physio-chemical and biological environments of the soil. The study has focused on efficient and sustainable production using bio-inoculants in tea cultivation, wherein chemical fertilizer can be reduced significantly to avoid further residual contamination in soil. Hence there is wide scope of extensively use of microbial community for betterment of eco-friendly cultivation of tea with sustaining production and economy of planters.

2.4 Project Objectives and Target Deliverables (as per the NMHS-Sanction Order)

The collection of rhizospheric soil samples from different zone of Darjeeling and Assam. From which 146 numbers of microbes have been isolated out of 26 from Darjeeling and 8 strains from Assam were selected after screening all the PGPR traits for further analysis. The entophytic and rhizospheric microbes isolated from Darjeeling and Assam are *Bacillus cereu, Yokenella regensburgei, Lelliottia amnigena, Bacillus vireti, Bacillus megaterium, Lactobacillus coleohominis, Bacillus safenesis, Bacillus licheniformis, Bacillus badius, Bacillus pumilus, Bacillus firmus, Bacillus mycoides, Lysinibacillus fusiformis, Lysinibacillus fusiformis, Serratia marcescens, Klebsiella aerogenes, Bacillus thuringiensis*.

The PGPR test of selected microbes have been conducted for phosphate solubilization, potassium solubilization, Siderophore and secondary metabolite such as hormones and enzyme. MALDI-TOF MS and 16S rRNA gene sequencing methodology were used for identification of bacterial isolates. The MALDI-TOF-MS identification have been done through direct spotting by each microscopically separated colony were picked and spotted on MALDI-TOF target plate and protein extraction by total protein of bacterial isolates were extracted and spotted on MALDI –TOF target plate. The DNA was isolated from the soil samples using the Nucleospin DNAkit. The amplicon libraries were prepared using the Nextera Index kit as per the 16S metagenomic sequencing library preparation protocol. For this, 16S rDNA specific primers were used for bacterial V3eV4.

The comparative evaluation under organic and inorganic inputs were conducted in the field trial at Assam and Darjeeling to access the impact of chemical residual effect overcome by applied PGPR. The quality and yield attributes were assessed using graded level of chemical fertilizer and organic manure with or without bio-inoculants in field trial at Darjeeling and Assam. The microbes were isolated which produces hormones along with plant growth promoting activity mainly phosphate solubilization, potassium solubilization, zinc solubilization.

The technology developed under the project was transferred directly to planters of Darjeeling and Assam. Demonstration cum training programme was organised at different places of Darjeeling hills. Field application technique according to slope gradient was also demonstrated. Developed consortium was distributed among participant for further development of microbial enriched compost/vermicompost at their own field

3 METHODOLOGIES/STARTEGY/ APPROACH – *supporting documents to be attached.*

3.1 Methodologies used (max. 500 words)

Isolation and screening of microbes

Survey the tea growing region and collected the sample from Darjeeling and Assam for sample collection. Total 146 microbes were isolated and screened out for their divergence and evolutionary relationship between morphological and biochemical attributes.

Enzyme activity assay: Molecular identification of the isolates:

Genomic DNA of the bacterial strains were extracted using the DNA isolation Kit (Bioline, Inc.,Taunton, MA, United States) and MALDI-TOF MS by instructions of manufacturers. The isolated DNA was subjected to Pcr amplification with a universal 16S primer set consisting of 27F and 1492R targeting the almost full length 16S rRNA gene.

Microbiome analysis of the rhizosphere:

Rhizosphere soil samples were harvested from the pots for the analysis of rhizosphere soil microbiomes. The DNA was isolated from the soil samples using the Nucleospin DNAkit. The amplicon libraries were prepared using the Nextera Index kit as per the 16S metagenomic sequencing library preparation protocol. For this, 16S rDNA specific primers were used for bacterial V3eV4.

Screening for PGP attributes:

Several microbes that have been isolated from the collected rhizospheric soil and were screened for Plant Growth Promoting Traits by Qualitative and Quantitative assay.

x. **Phosphate Solubilizing test**:
xi. **Siderophore Producing test:**
xii. **Indole Acetic Acid (IAA) test**:
xiii. **ACC deaminase assay**:
xiv. **Potassium solubilization:**
xv. **Zinc Solubilization:**
xvi. **Hydrogen cyanide (HCN) production:**
xvii. **Ammonia production:**
xviii. **Protease Activity:**

The fresh tea extracts prepared by using hot water extraction method 0.5 g. of each tea sample was taken in a beaker and 100 ml. of distilled water was added. The mixture was heated on a hot plate with continuous stirring using a magnetic stirrer at 30 0to 40C for 30 minutes. Then the extracts were cooled

and filtered through filter paper. The extracts were stored in polythene containers and used for further analysis.

i. **Determination of Total Phenolic content in Tea sample:**
ii. **Catalase activity:**
iii. **Estimation of glutathione (GSH)**
iv. **Estimation of superoxide dismutase (SOD, an antioxidant enzyme)**
v. **Determination of Soil enzyme activities-**
vi. **Dehydrogenase activity-**
vii. **Fluorescein diacetate hydrolysis (FDA):**

Field evaluation

The field experiment was conducted at Assam and Darjeeling for evaluation of consortium with and without fertilizer and organic manure.

Field Trial Experiment at Assam:

a. Control
b. Consortium
c. 25% Fertilizer + Consortium
d. 50% Fertilizer + Consortium
e. Fertilizer (only)

Field Trial Experiment at Darjeeling:

a. Control
b. 25% of recommended fertilizer + consortium
c. 50% of recommended fertilizer + consortium
d. 25% of recommended organic manure + consortium
e. 50% of recommended organic manure + consortium
f. Only consortium
g. Only recommended dose of fertilizer
h. Only recommended dose of organic manure

To observe the tea yield, plucking of the tender shoots by the skilled pluckers. Flush is the harvest season for teas. The tea yield per hectare was calculated with the help of the yield obtained in three plots (an area of 45 m2=area of one plot × replications=15 m^2×3) having 60 tea plants at Darjeeling site.

3.2 Data collected and Equipments utilized (max. 500 words)
Research activities under the project is only based on generated secondary data

3.3 Details of Field Survey conducted, if any (max 500 words)

Visited different locations of Darjeeling and survey the small tea growers (STG). Most of the STG are completely depend on cultivation of crop. The tea cultivation is an alternative cash crop of most of the small tea farmers in an opportunity for constant income generation for their livelihood. In changing climatic condition and occurrence of scarcity of water in from September to April in a year, production of traditional crop i.e. orange, ginger was declined, due to unavailable market, high cost of cultivation,

frequently incidents of disease and pest farmers switch to tea cultivation. So they approach for perennial tea crop in substitution of tradition crop. But during the discussion had with small tea growers they argue the status of local labor scarcity, education, lack of infrastructure for selling green leaves, the poor economic condition than the permanent worker in Tea Garden, suffered from low productivity under organic farming, rising input costs. Family person participated in cultural operation reduce poverty and compensate the scarcity of labour but their economic contribution is invisible. Darjeeling tea is under conversion of organic production so, the organic input for crop production is major concern in remote area of hills for small tea growers. Due to insufficient technical knowledge they brought the poor quality of organic resources with high cost and further visited different offices for organic certification. Due to unavailability of systematic market for selling of green leaves, small tea growers are financially weak have severely affected by declined price which was decided by big tea factories however cost of production was gradually increases.

During the visit, the team provided a detailed explanation of the work and objectives of the project. The organic tea practices currently being implemented in the Darjeeling hills face challenges in terms of managing organic resources, which can be quite costly. However, these difficulties can potentially be overcome by utilizing developed biofertilizer as a substitute. This project aims to explore the feasibility and effectiveness of using biofertilizer in organic tea production. The planters expressed great enthusiasm about the potential outcomes of the project and are eager to collaborate further by providing samples and adopting new technologies. Their willingness to cooperate indicates a strong commitment to improving the organic tea industry in the region. By embracing the use of biofertilizer, the planters hope to enhance the sustainability and productivity of their tea plantations.

One of the key advantages of using biofertilizer is the potential to reduce reliance on costly organic resources. This can significantly lower production costs, making organic tea more economically viable for both the planters and consumers. Additionally, biofertilizer offer a sustainable alternative to chemical-based fertilizers, minimizing the environmental impact of tea cultivation.

In conclusion of the visit provided an opportunity to discuss the challenges faced by organic tea practices in Darjeeling and Assam hills and explore potential solutions. The project's focus on utilizing developed biofertilizers offers a promising alternative to costly organic resources. The planters' enthusiasm and willingness to cooperate demonstrate their commitment to improving the industry. By embracing biofertilizers, the planters hope to enhance the sustainability and productivity of their tea plantations while reducing costs and minimizing environmental impact. Through continued collaboration and research, this project aims to pave the way for a more prosperous and sustainable future for the organic tea industry in the Darjeeling hills.

3.4 Strategic Planning for each activity with time frame (max. 200 words)

PROJECT ACTIVITIES	WORK UNDERTAKEN			
	Year 2019-22			
	1st year	2nd Year	3rd Year	6 Month ext.
Survey and sample collection				
Community DNA extraction				
Isolation of dominant members of microorganism from tea rhizosphere				
Screening for multiple plant growth promoting attributes				
Next generation sequencing of 16rRNA gene and ITS region				
Bioinformatics analysis to identify the tea rhizosphere microbiome				
Identification of newly isolated microorganism using MALDI-TOF MS and sequencing				

Development of bio-inoculant formulations based on tea core microbiome information, and strains with multiple plant growth promoting activities				
Evolution of microbial formulation in pot and field studies				
Survey will be conducted to access the socioeconomic condition in different regions				
Conducting seminars and meeting to educate the tea growers about sustainable means of tea cultivation				
Microbial formulations will be distributed among tea growers				
Training tea workers for production and application of biofertilizers				

4 KEY FINDINGS AND RESULTS – *supporting documents to be attached.*

4.1 Major Activities/ Findings (max. 500 words)

The field survey was organized with small tea growers and some Managers of Tea Estate in Darjeeling and Assam. A formal discussion had with tea growers and scientific personal were explained about the benefit and outcomes of the project.

The rhizospheric soil samples were collected from different tea estates of Assam and Darjeeling. The soil sample collected were further processed for isolation of bacteria by the serial dilution process. Several bacteria had been isolated. The isolates were screened for plant growth promoting attributes. The isolates that had generated the best PGP attributes were selected and 16s rRNA sequencing and indemnification were done.

The bacterial consortium of the isolates was developed and was applied on tea after field experiment at Darjeeling and Assam. The result obtained from the Pot trial and Field trial have shown how using bio-inoculants can greatly boost plant growth and increase agricultural yields. Bio-inoculants include beneficial bacteria that can improve plant vigour and productivity by stimulating hormones that promote plant growth, encouraging root formation, and increasing nutrient intake. It has been recorded that bio-inoculants improve the availability and uptake of nutrients by plants. Beneficial bacteria can fix atmospheric nitrogen, solubilize nutrients like phosphorus, and release growth-promoting compounds that make it easier to absorb nutrients. As a result, the nutritional condition of the soil is enhanced, and plants use nutrients more effectively.

After the completion of research work on indigenous microorganisms, the developed technology was directly transfer to small tea growers concerning adoption, use and impact of bio-inoculants available with us. The fundamental concern of the work is the adoption of recommended technology to replace the fertilizer application, maintained soil health and conservation, pest and disease control under organic farming and sustaining the economic production by using bio-inoculant.

Three hands-on training and demonstration programs were organized in Darjeeling Hills, with the aim of providing participants with practical knowledge and skills for further development and application of cultural practices in the field. These programs proved to be highly beneficial, with a total of 100 participants benefiting from them. Participants were introduced to various cultural techniques and methodologies during the training sessions. Through hands-on experience and demonstrations, they were able to gain a deeper understanding of these practices and their potential applications. The programs were designed to be interactive and engaging, allowing participants to actively participate and learn. Expert trainers and facilitators were present to guide the participants and provide them with the necessary support and guidance throughout the training sessions. They were also provided with culture kits, which included relevant materials and resources to further enhance their learning and development. The distribution of culture kits aimed to ensure that the participants continue their learning journey even after the programs have ended. These kits contained tools, materials, and resources that would enable the participants to implement the cultural practices in their work and contribute to their further development.

4.2 Key Results (max. 500 words in bullets covering all activities)

- Major 36 inoculants were assessed for various ecology of Darjeeling and Assam and their biochemical properties and colonization arrangement was assessed.
- Isolation, characterization of various rhizospheric and endophyte microbes for tea plant growth promoting traits like phosphate solubilization, growth hormone production, siderophore production, ACC deaminase production, ammonia production, hydrogen cyanide production along with various enzymatic activities was completed.

- Effect of isolated inoculants as biofertilizer was assessed at Darjeeling environmental condition with and without chemical fertilizer and organic manure at field level. Yield attributes and quality parameter was assessed as per treatment.
- The improve soil microbiome structure was resulted after the application of the bacterial consortium which enhanced the plant growth and soil health.
- The improve soil microbiome structure was resulted after the application of the bacterial consortium which enhanced the plant growth and soil health.
- The significant interaction between yield and treatments reveals that the application of graded level of recommended dose of fertilizer and recommended dose of organic manure in combination with consortium cause an increase in yield on a short term seasonal basis.
- The effect of application of consortium along with 50% recommended dose of fertilizer was observe increase in theaflavin and thearubigins content in all the three clones of tea I.e. AV2, T78 and P312 whereas polyphenol content was found higher with 50% organic manure along with consortium treated plot.
- All levels of the manure mixture with consortium were observed higher over control. It means that besides the optimized dose, the application of consortium also maintained the uptake of nitrogen, phosphorus and potash in Darjeeling condition.
- The environmental condition of experimental site was also assessed during experimentation period. About 90% of the rain was received from June to September and the rest are in autumn and winter season. The minimum and maximum temperature and humidity was observed in April to September which is the period of flush.
- In field study it is revealed that the indigenous inoculum showed a congenial soil environment for their growth and a clear synergistic effect in improving yield and quality tea cultivars under field condition. Thus the formulations could be used as efficient biofertilizer products capable of reducing chemical effect while improving growth and yield of low yielding tea field.
- The demonstration cum training of project formulation was held in October 2022 in Tukdah and Sittong of Darjeeling hills. Total 50 participants were benefitted from workshop/training.
- The consortium was distributed to some active member to produce enriched compost/vermicompost. The enriched compost enables the planter to fulfil the nutrient requirement of tea plant with a limited material available at their farm.
- The method and rate of consortium application in field and compost bed was also demonstrated and conducted by planters.
- The overall impact of use of consortium by small tea growers has increased their yield and soil health in organic manner may increase their financial stability in tea industry.

4.3 Conclusion of the study (max. 500 words in bullets)

In the conclusion of project on the creation and usage of bio-inoculants has proven to be a worthwhile undertaking with a range of applications and advantages in agricultural systems. The tea rhizosphere soil of Darjeeling and Assam was found to harbor a diverse range of microorganisms with multifarious PGP activity and biocontrol potential. These microorganisms have the ability to enhance plant growth and protect plants against various pathogens. Bio-inoculants have been studied and used to promote environmentally and economically sound agricultural practises, advancing sustainable agriculture practises. Several important outcomes have been proven by the application of bio-inoculants. Firstly, the use of bio-inoculants has reduced the need for synthetic fertilisers by increasing nutrient availability and uptake in plants. This reduction in chemical inputs not only lowers farmers' production costs, but it also reduces pollution and increases soil health and fertility. The bio-inoculants have been shown to be useful in controlling pests and illnesses in agricultural settings. These beneficial bacteria contribute to lessen the use of artificial pesticides by activating the plant's natural defence mechanisms. This reduces pesticides' detrimental effects on human health, beneficial insects, and overall ecosystem balance. The application of bio-inoculants has demonstrated potential for improving water-use efficiency, hence contributing to water conservation efforts in agricultural practises. Bio-inoculants help plants access water more effectively by boosting root development and nutrient uptake, minimising the need for excessive irrigation and conserving valuable water resources.

The inoculum isolated from the tea rhizosphere soil of Darjeeling and Assam have isolated are observed to multifarious PGP activity and biocontrol potential. Field studies on application of the isolated microbiome was conducted to the indigenous location of Darjeeling and Assam. The outcome of experiment utmost importance in deciding the feasibility of the technique to apply in the planter’s field. Investigation of region-specific tea indigenous microorganisms having PGP activity and biocontrol prospective is imperative to achieve suitable productivity by maintaining soil health, fertility management and suppress the residual chemical effect on plant growth.

Thus, the inoculum isolated from the tea rhizosphere soil of Darjeeling and Assam exhibited multifarious PGP activity and biocontrol potential. The field study conducted in the indigenous locations of Darjeeling and Assam demonstrated the effectiveness of the isolated microbiome in improving plant growth and controlling plant diseases. These findings hold great importance in deciding the feasibility of implementing this technique in tea plantations.

5 OVERALL ACHIEVEMENTS – *supporting documents to be attached.*

5.1 Achievement on Project Objectives/ Target Deliverables (max. 500 words)]

- Major 36 inoculants were assessed for various ecology of Darjeeling and Assam and their biochemical properties and colonization arrangement was assessed.
- Isolation, characterization of various rhizospheric and endophyte microbes for tea plant growth promoting traits like phosphate solubilization, growth hormone production, siderophore production, ACC deaminase production, ammonia production, hydrogen cyanide production along with various enzymatic activities was completed.
- The method and rate of consortium application in field and compost bed was demonstrated and conducted by planters. Consortium application refers to the practice of combining multiple species of plants in a given field or compost bed. This method is commonly used in agriculture to enhance soil fertility, increase crop productivity, and promote ecological balance.
- By utilizing consortium application, planters can achieve several benefits. Firstly, the different plant species in the consortium can contribute to nutrient cycling, where each species takes up different nutrients from the soil and releases them back into the environment through their roots or decomposing plant matter. This helps to maintain a balanced nutrient supply and reduces the need for synthetic fertilizers. Secondly, consortium application can enhance soil structure and water retention capacity. Different plant species have different root structures that can penetrate the soil at different depths, improving soil aeration and water infiltration. This can help prevent soil erosion and promote healthier plant growth.
- The use of bio-inoculants has gained significant attention in recent years as an alternative to chemical fertilizers. Bio-inoculants, such as isolated microbes, offer a sustainable and eco-friendly approach to enhance crop productivity.
- The field-level experiment was conducted in Darjeeling and Assam, two regions known for their distinct environmental conditions. The study employed a randomized complete block design with several treatment combinations. The treatments included the use of isolated inoculants alone, isolated inoculants with chemical fertilizer, isolated inoculants with organic manure, chemical fertilizer alone, organic manure alone, and control (no biofertilizer, chemical fertilizer, or organic manure).
- Various yield attributes, including plant height, number of branches, number of flowers, and number of fruits, were recorded for each treatment. Quality parameters such as fruit weight, fruit size, and nutritional content were also assessed. Data were analysed using appropriate statistical methods to determine the effects of the different treatments.
- The observed positive effects of isolated inoculants as bio-inoculants in both Darjeeling and Assam suggest their potential as a sustainable agricultural practice. The incorporation of isolated inoculants in crop production systems can reduce the reliance on chemical fertilizers, thus minimizing the negative environmental impacts associated with their usage. The synergistic effects of isolated inoculants with chemical fertilizer and organic manure highlight the importance

of integrated nutrient management strategies for maximizing crop productivity and maintaining soil health.

- The present study demonstrates that the use of isolated inoculants as biofertilizer can significantly improve yield attributes and quality parameters of crops in Darjeeling and Assam environmental conditions. The combined application of isolated inoculants with chemical fertilizer and organic manure showed the most promising results, indicating the potential for integrated nutrient management practices in sustainable agriculture. These findings contribute to the growing body of knowledge on bio-inoculants and their role in enhancing crop productivity while minimizing environmental impacts.
- Hands-on training and demonstration programs were organized in Darjeeling and Assam Hills, with the aim of providing participants with practical knowledge and skills. These programs proved to be highly beneficial, with a total of 80 participants benefiting from them. Participants were introduced to various cultural techniques and methodologies during the training sessions. Through hands-on experience and demonstrations, they were able to gain a deeper understanding of these practices and their potential applications.

5.2 Interventions (max. 500 words)

The developed bio-inoculants comprised of bacteria from core microbiome that colonise rhizosphere. They produce plant growth hormones, solubilize nutrients, control disease, and promote root formation, among other advantages for plants. Plant growth and productivity are enhanced by the use of PGPR inoculants. The use of bio-inoculants in agricultural systems has gained popularity due to their ability to increase crop output, reduce environmental impact, and improve soil health. However, sufficient understanding of the individual plant-microbe interactions, optimal formulation and application methods, and careful consideration of site-specific circumstances are required for successful implementation. Research and development activities are continuing to broaden our understanding of various microorganisms and their potential applications as bio-inoculants. This work provided the product to the small scale tea grower for performance evaluation and had sufficient shelf life to provide customised solutions for tea growing in different environments, ultimately contributing to sustainable and environmentally friendly agricultural practises.

The findings were significantly evaluated in field experiment of native location of isolated microbes in Assam and Darjeeling. The concrete outcome of research was consortium which has handed over to the small tea growers directly to multiplication and application of bio-inoculum at their own field by producing enriched compost/vermicompost. In the history of tea industry of Darjeeling and Assam, it was the first time to directly delivered the bio-inoculants to planters without any cost. The planters were keenness to adopt and use the bio-inoculant developed under the project. Enriched manure will be compensating the

additional nutrient requirement of plant which reduces their dependency on chemical and other costly market input. The microbes hastened the decomposition of farm waste enable the farmers to produce sufficient quantity of farm compost. The use of bio-inoculated compost/vermicompost in the field nourishes the soil health eco-friendly. The sustainable eco-friendly production may increase the extra earning of planters have direct impact of their family economic growth. About 100 small tea growers of different location of Assam and Darjeeling were directly benefited from the bio-inoculant developed under the project and they are requested again to conduct this type of event in future for more benefit.

5.3 On-field Demonstration and Value-addition of Products, if any (max. 500 words)

The demonstration of project formulation took place in June 2021 at Pokharibong and in October 2022 in Tukdah and Sittong of Darjeeling hills. These events served as platforms for showcasing the project and its goals to the participants. During the project, the participants underwent training in a classroom setting to familiarize themselves with the project's objectives and methodologies. They were equipped with the necessary knowledge and skills to implement the project effectively.

One of the key activities of the project involved the application of inoculants in the field. The participants themselves carried out this task, putting into practice what they had learned during the training. The workshop provided an opportunity for the members to discuss and share their insights on the project. They reached a consensus that the project focused on harnessing the potential of beneficial microorganisms to enhance nutrient mobilization for plants, particularly nitrogen phosphorus and potash. This approach aimed to improve the overall health and productivity of the plants.

The bio-inoculants prepared in the laboratory with the rhizosheric bacteria of Tea was demonstrated and distributed among the tea workers of Rosekandy Tea Estate, Cachar, Assam. Through a hands-on training, the tea workers were educated about the benefits of using the consortium which enhance the soil fertility along with the plant growth. Implementing bacterial consortium increases the nutrient uptake by the plants, improves the plant growth promoting attributes such as conversion of inorganic phosphorous to solubilized form, nitrogen fixation, potassium solubilization etc. The bio-inoculants developed by the soil and Environmental Microbiology Laboratory (SEML), Assam University, Silchar under NMHS Project, which is purely organic product. The consortium distributed to the tea planters helped them to achieve the nutrient requirement for the plant for its growth. The tea workers have implemented the bio-inoculants in the field.

Overall, the project demonstration and workshop were essential in showcasing the potential benefits of utilizing beneficial microorganisms in agriculture. The participants gained valuable knowledge and practical experience, which will contribute to the success of future endeavours in this field. The utilization of consortium by small tea growers has proven to be an effective strategy in increasing their yield and improving soil health. This, in turn, contributes to their financial stability within the tea industry. By conserving and enriching the inoculants through the transfer of enriched compost and potentially

providing more consortium in the future, tea growers can continue to reap the benefits of this innovative approach to tea cultivation in hilly region of Darjeeling and Assam.

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5.4 Green Skills developed in in State/ UT (max. 500 words)

The green revolution brought impressive gain in food production by using chemical fertilizers without insufficient concern for sustainability. Dependence on chemical fertilizer for future agriculture growth would mean further loss in soil quality and surroundings environment. The availability and affordability of fossil and fuel on farm have ensured to increase the production and productivity through in-situ management of farm input. The awareness events enable the planter to use the developed bio-inoculant for production of enriched compost by available waste material on farm. This technical knowledge enables to manage cost effective input preparation by their own along with management of waste material.

The use of bio-inoculants in tea gardens helped to create "green skills" or ecologically friendly practises that encourage sustainable agriculture. Bio-inoculants have the potential to improve nutrient availability and uptake in tea plants, lowering the need for synthetic fertilisers. Beneficial bacteria in bio-inoculants can help with nutrient cycling, nitrogen fixation, phosphorus solubilization, and the availability of other critical nutrients. The use of bio-inoculants encourages the fertility and health of the soil. The beneficial microorganisms in bio-inoculants enhance soil water-holding capacity, raise soil organic matter content, and improve soil structure. As a result, erosion is decreased, nutrient cycling is improved, and soil health is increased. This bio-inoculants can strengthen the plant's built-in defences and lower the likelihood of pests and illnesses. This bio-inoculants assist in reducing the reliance on chemical pesticides in tea gardens, minimising environmental pollution, and maintaining biodiversity by fostering a healthier plant and enhancing the plant's immune response. Farmers can acquire environmentally friendly skills that support sustainable practises, minimise chemical inputs, improve soil health, conserve water, and mitigate climate change by implementing bio-inoculants in tea gardens. These environmentally friendly methods help tea producing systems remain resilient and generally sustainable.

5.5 Addressing Cross-cutting Issues (max. 200 words)

To improve the effectiveness and efficiency of bio-inoculants, continuous research and development activities are required. This entails researching diverse strains of helpful microbes, optimising their formulations, and determining their specialised usage in different crops and environmental situations. For farmers, the economic viability of utilising bio-inoculants is an important factor. Bio-inoculants can be made more financially accessible and appealing to farmers by cost-effective production methods, pricing structures, and incentives, ensuring their widespread adoption and long-term sustainability. It is critical to assess the potential environmental implications of bio-inoculant application. This includes assessing their effects on soil biodiversity, ecosystem functioning, and non-target organisms to ensure that the application of bio-inoculants poses no unexpected environmental concerns. Addressing these

intersecting concerns is critical for the successful development, adoption, and long-term use of bio-inoculants in agriculture. By taking these elements into account, we can fully utilise the potential of bio-inoculants to promote sustainable farming practises, increase tea production, and reduce the environmental footprint of agricultural systems.

6 PROJECT'S IMPACTS IN IHR – *supporting documents to be attached.*

6.1 Socio-Economic impact (max. 500 words)

The application of biofertilizer has brought some changes on socioeconomic condition of the small tea growers of Darjeeling. Darjeeling tea has almost converted to organic and bought leaf factory has clear cut instruction to bought organic produce only. Under this situation requirement of organic input to supply the nutrient demand of tea crop has fulfilled by using this bio-inoculant. The planters have started to produce their own enriched compost and freely uses in tea and other cash crops to increase their production and productivity. Easily available consortium saving time and money and reduction of risk of chemical residual effect in their organic product.

The bio-inoculants distributed to the tea growers has brought a change in the socioeconomic conditions of the tea growers in Assam. The tea growers in Assam almost uses the chemical fertilizers which hamper the growth of plants and soil conditions. Therefore, the application of the bio-inoculants will give an opportunity for them to replace the harmful chemical with time. The demand for the organic products, in recent times by the people has provide a new insight for the use of bio-inoculants which is environmentally friendly. The use of the biofertilizer had brought a positive impact on the tea growers. Increased in product will enhance food security, generates income for farmers, and contributes to economic growth. The use of bio-inoculants aligns with sustainable agricultural practices and environmental conservation. By reducing the dependency on chemical inputs, bio-inoculants help minimize soil and water pollution, protect biodiversity, and preserve ecosystem health. The adoption of bio-inoculants can contribute to rural development by generating employment opportunities. The production, distribution, and application of bio-inoculants create jobs along the value chain, supporting local economies.

Eco-friendly cultivation of tea had a positive impact on the health of family members and time saving for collection of organic inputs from different places. Organic produce may get maximum market value and enables the small grower to negotiate for the price for their organic product. The use of biofertilizer in their cultivation reduced expenses and time for collection of organic input for nutrient requirement in cultivation of tea. Furthermore, the use of bio-inoculants can diversify agricultural practices, enabling farmers to explore new markets and value-added products, thereby enhancing rural livelihoods.

6.2 Impact on of Natural Resources/ Environment (max. 500 words)

The conservation agriculture practice is an essential approach that seeks to address the current situation in agriculture. With the increasing need for sustainable and environmentally-friendly farming methods, there has been a global force to promote safe ecology practices in agriculture. One such practice is the use of bio-inoculum, promote sustainable farming practices by reducing the negative environmental impacts associated with chemical fertilizers. Chemical fertilizers often lead to nutrient imbalances, soil degradation, and water pollution. On the other hand, bio-inoculum support the natural processes in the soil ecosystem, maintaining its balance and preventing the accumulation of harmful substances. which has gained momentum at a fast pace.

This bio-inoculants application played a significant role in improving soil health and fertility. It enhanced nutrient availability, promote organic matter decomposition, and improve soil structure. By enriching the soil with beneficial microorganisms, bio-inoculants contributed to the sustainable use of natural resources by ensuring optimal soil conditions for plant growth and nutrient cycling. By improving nutrient availability and recycling, bio-inoculant minimized nutrient losses and contributed to the conservation of natural resources. The application of these bio-inoculants back to the tea soil further enhanced the soil fertility and improves the growth of the plant.

The bio-inoculant used in this study was prepared through a meticulous screening process. The aim was to identify efficient indigenous microorganisms that were isolated from native Camellia sinensis plants found in the regions of Darjeeling and Assam. These microorganisms have been carefully selected for their beneficial properties and are considered natural, organic, and biodegradable. As a result, they are environmentally friendly and pose no harm to the ecosystem. One of the key advantages of using these indigenous microorganisms is their ability to improve soil fertility. By introducing them into the soil, they enhance its nutrient content and overall quality. This, in turn, creates a more favorable environment for plants to grow and thrive. By utilizing the bio-inoculant, farmers and gardeners can reduce their dependence on synthetic chemicals. The microorganisms within the inoculant help to enhance the natural processes within the soil, reducing the need for artificial fertilizers and pesticides. This not only benefits the plants and the environment but also contributes to sustainable agricultural practices.

In summary, the bio-inoculant prepared from indigenous microorganisms isolated from Camellia sinensis plants of Darjeeling and Assam offers numerous advantages. Its natural, organic, and biodegradable nature makes it environmentally friendly. Furthermore, it improves soil fertility, promotes plant growth, and reduces the reliance on synthetic chemicals. By harnessing the power of these microorganisms, farmers and gardeners can enhance their agricultural practices while minimizing negative impacts on the environment.

6.3 Conservation of Biodiversity/ Land Rehabilitation in IHR (max. 500 words)

In recent years, there has been a notable shift in agricultural practices towards an old-age approach known as conservation agriculture. This method combines traditional tools and techniques with modern scientific principles, emphasizing the importance of sustainable farming and environmental preservation. By incorporating age-old practices with advancements in science, conservation agriculture embraces a holistic approach that emphasizes soil health, biodiversity, and water conservation. By adopting conservation agriculture, farmers aim to minimize ecology disturbance, soil erosion, preserve biodiversity & organic matter, and enhance natural processes leading to improved productivity and long-term sustainability.

In the process of metabolizing nutrients, bio-inoculants also generate waste products that need to be eliminated from their cells. By efficiently removing waste, microbes maintain the balance of their internal environment and ensure proper functioning of their cellular processes. Bio-inoculums are highly adaptable organisms that can sense and respond to changes in their environment. They possess mechanisms that allow them to detect and react to factors such as temperature, pH, light, and the presence of other organisms. These responses can range from simple behavioural changes to complex regulatory processes that enable them to survive and thrive in different conditions. The biological systems of microbes share several characteristics that are essential for their survival and proliferation. The ability to reproduce, ingest or assimilate food substances, metabolize them for energy and growth, excrete waste products, and respond to environmental changes are all fundamental to the life processes of microbes. Understanding these shared traits helps us appreciate the remarkable adaptability and resilience of these microscopic organisms.

The application of the bio-inoculants plays a crucial role in promoting the conservation of biodiversity by enhancing soil fertility and supporting the growth and development of plants. We characterized the complete microbiome of Darjeeling and Assam tea, with structure and functional detail of microbial diversity using molecular approaches. This information was used to design and develop the microbial consortium based bio-inoculant.

Field studies have shown that indigenous inoculum creates a favorable soil environment for its growth. This indigenous inoculum also exhibits a clear synergistic effect, enhancing the yield and quality of tea cultivars under field conditions. As a result, these formulations can be utilized as highly efficient biofertilizer products. By utilizing these microbes, the reliance on chemical fertilizers can be reduced while simultaneously improving the growth and yield of low-yielding tea fields. This has significant implications for sustainable agricultural practices, as it promotes both environmental and economic benefits. The use of bio-inoculants not only minimizes the negative impact of chemical fertilizers on the environment but also enhances the overall productivity of tea cultivation. By harnessing the power of

indigenous inoculum, tea farmers can optimize their crop yield and quality while adhering to environmentally friendly farming practices.

6.4 Developing Mountain Infrastructures (max. 200 words)
Not related to with the objectives of project

6.5 Strengthening Networking in State/ UT (max. 200 words)
Not related to with the objectives of project

7 EXIT STRATEGY AND SUSTAINABILITY – *supporting documents to be attached.*

7.1 Utility of project findings (max. 500 words)

Environmentally beneficial, the bio-inoculants were developed. The soil from the tea rhizosphere was used to isolate the bacteria that was used to generate bio-inoculants. The use of this bacterial consortia boosts plant growth-promoting processes such the solubilization of potassium, nitrogen fixation, and the conversion of inorganic phosphorous to a soluble form. The utilization of bio-inoculants brings several benefits to the tea plantations. One of the significant advantages is their ability to reduce soil and water pollution. By using bio-inoculants, the tea growers can minimize the use of chemical fertilizers and pesticides, thus reducing the pollution of soil and water resources. Another crucial aspect of utilizing bio-inoculants is the protection of biodiversity.

These bio-inoculants promote the growth of beneficial microbes in the soil, which in turn enhances the overall biodiversity of the tea plantation. By fostering a healthy and diverse ecosystem, the tea growers contribute to the preservation of various plant and animal species within their surroundings. Additionally, the use of bio-inoculants helps maintain the sustainability of tea plantations. By enhancing soil fertility and promoting nutrient cycling, bio-inoculants enable the tea plants to grow in a more sustainable manner. This ensures that the tea growers can continue to harvest high-quality tea for years to come while minimizing the negative impact on the environment.

Bio-inoculants are beneficial microorganisms that have the potential to enhance soil fertility and plant growth. By harnessing the power of these microorganisms, planter's communities can increase their income and improve their overall well-being. The use of bio-inoculants offers several advantages, including the recycling of waste products and the production of enriched organic manure. Farm activities generate a significant amount of waste, including crop residues, animal manure, and other organic materials. Rather than letting these waste products go to waste, planter's communities can utilize bio-inoculants to convert them into enriched organic manure. This process involves the decomposition of compost, facilitated by the microorganisms present in the bio-inoculants. Composting at high altitudes in hilly regions can be challenging due to the cold climate and limited microbial activity. However, bio-inoculants have shown great potential in overcoming these challenges. The microorganisms in bio-inoculants accelerate the decomposition process, even in colder climates, making it easier to produce

high-quality compost in hilly regions. Bio-inoculant are considered to be a low cost environment friendly alternative source of organic nutrient supply to the plant. The use of bio-augmented compost improves physiological, chemical and biological environment of soil, enhance photosynthetic capacity of tea, develop resistance in plant to disease and pest. It maintains soil fertility in sustainable manner for longer period, reduces the dependency on hazardous chemical fertilizer, helps to control soil erosion in hilly region and finally provided the eco-friendly environment and healthy produce.

7.2 Other Gap Areas (max. 200 words)

The practices of good agricultural are work as much as possible with in closed system with regard to organic matter and nutrient elements. Soil samples have been collected from planter's field and analyzed as per recognized soil fertility analytical procedures to have accurate soil fertility information for each field management unit. Estimated the yield potential for each field based on soil productivity and intended management and then fix up the yield target. On the basis of requirement, the doses of the nutrients to be supplied through bio-augmented organic manure considering indigenous nutrient supply. The planter opted the good agricultural practices related to maintain soil fertility, nutrient management, soil moisture, soil reaction and soil conservation. The use of organic mulches to field and collection of pruning litter from farm and cowshed to utilized for preparation of enriched compost using bio-inoculum on own farm. The planters were suggested to use leguminous species shade trees which will help to improve biological nitrogen fixation and availability to the plant. Grow in situ green manuring by growing Guatemala or legumes like cowpea, sun hemp in vacancies to add organic matter and other nutrients in the soil.

7.3 Major Recommendations/ Way Forward (max. 200 words)

This study investigated the diversity of microbes in caves, which could hold a great deal of potential for the development of new bioactive compounds. The discovery of new active metabolites from culturable microorganisms has significant implications for tea fields, including stress tolerance, soil health, fertility management, biocontrol of pest and disease. Furthermore, they can provide valuable insights into the complex interactions between microorganisms and their environment, contributing to our understanding of microbial ecology and evolution in conventional cultivation of tea in hilly region.

The next stage of this work should be designed for the application of this technology on a large scale and develop a chemical free Himalayan ecosystem. For the subsistence of Himalayan biodiversity, encouraging sustainable use this kind of bio-inoculant is essential. Promoting sustainable agriculture, organic farming, and agroforestry can ease the strain on natural ecosystems.

The application of beneficial microbes, including bacteria and fungi, to tea cultivation systems holds immense potential for increasing crop productivity and addressing the problem of low productivity. By improving soil health, enhancing biodiversity, and promoting efficient nutrient use, these microbes offer a

sustainable and environmentally friendly solution. As farmers hold this innovative technique, they can contribute to the development of a more resilient and productive agricultural sector.

7.4 Replication/ Upscaling/ Post-Project Sustainability of Interventions (max. 500 words)

8 REFERENCES/BIBLIOGRAPHY

Sharma A, Shankhdhar D, Shankhdhar SC (2014) Growth promotion of the rice genotypes by PGPRs isolated from rice rhizosphere. J Soil Sci Plant Nutr 14:505–517

Jintu Dutta and Debajit Thakur (2017) Evaluation of multifarious plant growth promoting traits, antagonistic potential and phylogenetic affiliation of rhizobacteria associated with commercial tea plants grown in Darjeeling, India . PLoS ONE 12(8):e0182302.

Bagyaraj DJ, Ashwin R (2017) Soil biodiversity: role in sustainable horticulture. Biodivers Hortic Crops 5:1–18

9 ACKNOWLEDGEMENTS

Tea Board India is immensely grateful to the Ministry of Environment, Forest and Climate Change (MoEF&CC), Government of India, and G.B. Pant National Institute of Himalayan Environment & Sustainable Development (GBPNIHESD) for their unwavering support and funding provided towards the implementation of the project under the National Mission for Himalayan Studies (NMHS). The financial assistance provided by these esteemed organizations has not only contributed to the advancement of scientific knowledge but has also helped in the protection and preservation of this fragile ecosystem.

We would like to extend our heartfelt thanks to the Deputy Chairman, Secretary, and Director of Research of Tea Board for their invaluable support, guidance, and unwavering enthusiasm throughout this project. Their contributions have been instrumental in the successful completion of this endeavor, and we are truly grateful for their continued support.

We extend our sincere thanks to the colleague of Tea Board India, Assam University, and NCCS Pune for their invaluable contributions to this project. Their technical expertise, financial support, and constant guidance have been instrumental in its success.

We express our heartfelt gratitude to the community people across the two States for their invaluable support, cooperation, and unwavering enthusiasm throughout this project. Without their collective efforts, this endeavor would not have been possible.

We are also grateful to the dedicated teams in the technical and financial departments, as well as the support staff, who have been a constant source of assistance and guidance at every step of this project. Their unwavering commitment and hard work have played a crucial role in overcoming challenges and ensuring its smooth execution.

APPENDICES

Appendix 1 – Details of Technical Activities
Appendix 2 – Copies of Publications duly Acknowledging the Grant/ Fund Support of NMHS
Appendix 3 – List of Trainings/ Workshops/ Seminars with details of trained resources and dissemination material and Proceedings
Appendix 4 – List of New Products (utilizing the local resources like NTFPs, wild edibles, bamboo, etc.)
Appendix 5 – Copies of the Supporting Materials like Manual of Standard Operating Procedures (SOPs) developed under the project
Appendix 6 – Details of Technology Developed/ Patents filled, if any
Appendix 7 – Any other

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Annexure-I

Consolidated and Audited
Utilization Certificate (UC) and Statement of Expenditure (SE)

For the Period: 21.01.2019 to 31.10.2022

1.	Title of the project/Scheme/Programme:	**Development of microbial inoculant to improve growth and productivity of Darjeeling and Assam**
2.	Name of the Principle Investigator & Organization:	**Rajesh Kumar Chauhan** **DTRDC, Tea Board, Darjeeling** **Dr. Biswajit Bera** **Ex. Director Research** **Tea Board** **Prof. Piyush Pandey** **Assam University** **Praveen Rahi** **NSSC Pune**
3.	NMHS-PMU, G.B. Pant National Institute of Himalayan Environment, Kosi-Katarmal, Almora, Uttarakhand Letter No. and Sanction Date of the Project:	**GBPNI/NMHS-2018-19/MG 1** **dated: 21-01-2019**
4.	Amount received from NMHS-PMU, G.B. Pant National Institute of Himalayan Environment, Kosi-Katarmal, Almora, Uttarakhand during the project period (Please give number and dates of Sanction Letter showing the amount paid):	**GBPNI/NMHS-2018-19/MG 1** **dated 21.01.2019** **Amount Rs. 3492000.00** **GBPNI/NMHS-2018-19/MG/01/217/220/51** **Dated: 06.06.2022** **Amount Rs. 19,58,402.00**
5.	Total amount that was available for expenditure (Including commitments) incurred during the project period:	**Rs. 5,47,743.25**
6.	Actual expenditure (excluding commitments) incurred during the project period:	**Rs.50,73,115.47**
7.	Unspent Balance amount refunded, if any (Please give details of Cheque no. etc.):	
8.	Balance amount available at the end of the project:	**Rs. 5,47,743.25**
9.	Balance Amount:	**Rs. 5,47,743.25**

10.	Accrued bank Interest:	**Rs. 1,72,728.00**

Certified that the expenditure of **Rs. 50,73,115.47 (Rupees Fifty Lakh Seventy Three Thousand One Hundred Fifteen and Fourty Seven Paisa Only)** mentioned against Sr. No. 6 was actually incurred on the project/scheme for the purpose it was sanctioned.

Date:

(Signature of Principal Investigator) (Signature of Registrar/ Finance Officer) (Signature of Head of the Institution)

OUR REF. No.

ACCEPTED AND COUNTERSIGNED

Date:

COMPETENT AUTHORITY
NATIONAL MISSION ON HIMALAYAN STUDIES (GBP NIHE)

Statement of Consolidated Expenditure

[Tea Board of India]

Statement showing the expenditure of the period from : 01.04.2022 to 31.10.2022
Sanction No. and Date : Ref: GBPNI/NMHS-2018-19/MG 1 Dated 21.01.2020

1. Total outlay of the project : Rs. 73,60,640.00

2. Date of Start of the Project : May 2019 (Fund received Fellow joined)

3. Duration : 3 years (Nine Month extension w.e.f 01.02.2022)

4. Date of Completion : October 2022 (Ref. GBPNIiNMHS-2018-19/MG1 217/220/243 dated 01.02.2022)

a) Amount received during the project period : Rs. 54,50,602.00

b) Total amount available for Expenditure : Rs. 5,47,743.25

S.No.	Budget head	Amount Carried forward	Amount received (FY-20-21)	Other receipts/ interest earned	Amount received+ amount carried forward (2+3+4)	Expenditure	Amount Balance/ excess expenditure
	1	2	3	4	5	6	7
1	Salaries	(7,90,667.00)	7,32,000.00	0.00	(58,667.00)	6,09,490.00	(6,68,157.00)
2	Permanent Equipment Purchased (Item-wise	2,70,370.00	0.00	0.00	2,70,370.00	0.00	2,70,370.00
3	Consumables	97,729.97	8,66,402.00	0.00	9,64,131.97	5,07,468.00	4,56,663.97
4	Contingency	83,580.00	1,30,000.00	0.00	2,13,580.00	74,416.34	1,39,163.66
5	Travel	19,185.00	1,80,000.00	0.00	1,99,185.00	19,939.38	1,79,245.62
6							
7							
8							
9							
10	Institutional charges	0.00	50,000.00	0.00	50,000.00	50,000.00	0.00
11	Accrued bank Interest	1,33,598.00	0.00	1,72,728.00	1,72,728.00		1,72,728.00
12	**Total**	**(1,86,203.75)**	**19,58,402.00**	**1,72,728.00**	**18,11,327.97**	**12,61,313.72**	**5,50,014.25**
13	Amount allowed to be Carried forward to the next financial year						0.00

Certified that the expenditure of **Rs.1261313.72 (Rupees:Twelve Lakh Sixty One Thousand Three Hundred Thirteen and Seventy Two paisa oby)** mentioned against Sr. No.12 was actually incurred on the project/ scheme for the purpose it was sanctioned.

Date:

(Signature of Principal Investigator)	(Signature of Registrar/ Finance Officer)	(Signature of Head of the Institution)

OUR REF. No.

ACCEPTED AND COUNTERSIGNED

Date:

COMPETENT AUTHORITY
NATIONAL MISSION ON HIMALYAN STUDIES (GBP NIHE)

Annexure-II

Consolidated Interest Earned Certificate

This is to certify that we have received the total amount Rs. ……../- (Rupees ……only) from bank as Interest Income during the project period, the details are as follows:

Financial year 2019-20:	Rs. 87,279.00
Financial Year 2020-21:	Rs. 42,051.00
Financial Year 2021-22:	Rs. 11,268.00
Financial Year 2022-23:	Rs. 32,130.00
Total Amount :	**Rs. 1,72,728.00**

Annexure-III

Consolidated Assets Certificate

Assets Acquired Wholly/ Substantially out of Government Grants

(Register to be maintained by Grantee Institution)

Name of the Sanctioning Authority: G.B. Pant National Institute of Himalayan Environment and Sustainable Development

1. Sl. No. _____
2. Name of Grantee Institution: Tea Board of India
3. No. & Date of sanction order: GBPNI/NMHS-2018-19/MG 1, Dated: 21-01-2019
4. Amount of the Sanctioned Grant: ₹73,60,640.00 (Rupees Seventy-Three Lakh Sixty Thousand Six Hundred Forty Only)
5. Brief Purpose of the Grant: To explore the functional roles of specific microorganisms and their interactions with tea plants. Understanding these relationships can aid in the development of targeted strategies to enhance tea production, planter's economy, and ensure the sustainability of tea cultivation in the Darjeeling and Assam regions.
6. Whether any condition regarding the right of ownership of Govt. in the property or other assets acquired out of the grant was incorporated in the grant-in-aid Sanction Order: _____
7. Particulars of assets actually credited _____ or acquired_____
8. Value of the assets as on _____
9. Purpose for which utilised at present _____
10. Encumbered or not _____No_____
11. Reasons, if encumbered _____
12. Disposed of or not _____
13. Reasons and authority, if any, for disposal_____
14. Amount realised on disposal _____No_____

Any Other Remarks: _____

(PROJECT INVESTIGATOR) **(FINANCE OFFICER)**

(Signed and Stamped) **(Signed and Stamped)**

(HEAD OF THE INSTITUTION)

(Signed and Stamped)

Annexure-IV

List or Inventory of Assets/ Equipment/ Peripherals

S. No.	Name of Equipment	Quantity	Sanctioned Cost	Actual Purchased Cost	Purchase Details
1	Spectrophotometer	1	3.00Lakh	2.50 Lakh	Used for estimation of nutrient content in leaves and soil.
2	Oxford Nanopore sequencer with accessories	1	6.00Lakh		To determine the sequence of DNA/RNA bases.
3	High-end workstation	1	4.00Lakh		

(PROJECT INVESTIGATOR)

(Signed and Stamped)

(FINANCE OFFICER)

(Signed and Stamped)

(HEAD OF THE INSTITUTION)

(Signed and Stamped)

Annexure-V

Letter of Head of Institution/Department confirming Transfer of Equipment Purchased under the Project to the Institution/Department

To,

The Convener, Mountain Division
Ministry of Environment, Forest & Climate Change (MoEF&CC)
Indira Paryavaran Bhawan
Jor Bagh, New Delhi-110003

Sub.: Transfer of Permanent Equipment purchased under Research Project titled "...." funded under the NMHS Scheme of MoEF&CC – reg.

Sir/ Madam,

This is hereby certified that the following permanent equipment purchased under the aforesaid project have been transferred to the Implementing Organization/ Nodal Institute after completion of the project:

1. Spectrophotometer
2. Oxford Nanopore sequencer with accessories
3. High-end workstation
4. .....
5. .....
6. .....
7. .....

Head of Implementing Organization:
Name of the Implementing Organization: Tea Board of India
Stamp/ Seal:
Date:

Copy to:

1. The Nodal Officer, NMHS-PMU, National Mission on Himalayan Studies (NMHS), G.B. Pant National Institute of Himalayan Environment (NIHE), Kosi-Katarmal, Almora, Uttarakhand-263643

Annexure-VI

Details, Declaration and Refund of Any Unspent Balance

Please provide the details of refund of any unspent balance and transfer the balance amount through RTGS (Real-Time Gross System) in favor of **NMHS GIA General** and declaration on the official letterhead duly signed by the Head of the Institution.

Kindly note the further Bank A/c Details as follows:

Name of NMHS A/c:	NMHS GIA General
Bank Name & Branch:	Central Bank of India (CBI), Kosi Bazar, Almora, Uttarakhand 263643
IFSC Code:	CBIN0281528
Account No.:	3530505520 (Saving A/c)

In case of any queries/ clarifications, please contact the NMHS-PMU at e-mail: nmhspmu2016@gmail.com