National Mission on Himalayan Studies (NMHS)

HIMALAYAN RESEARCH FELLOWSHIP

(PRO FORMA FOR THE FINAL TECHNICAL REPORT)

[Reporting Period: *April 2018 to* April 2021]

NMHS-Fellowship FINAL TECHNICAL REPORT (FTR)

1. Fellowship Grant Information and Other Details

NMHS Fellowship Grant ID:	GBPNI/NMHS-2017-18/HSF-07, dated: 28.03.2018
Name of the Institution/ University:	SIKKIM UNIVERSITY
No. of Himalayan Research/Project Associates:	01
No. of Himalayan Junior Research/Project Fellows:	03

2. Fellowship Description at H-RA Level

Himalayan Research Associates (H-RAs)

H-RAs Profile Description:

S. No.	Name of RA/ SRF	Date of Joining	Research Title	Name of the PI and Designation	Qualification
1.	Mingma Thundu Sherpa	30.11. 2018	Development of microbial inoculums for organic farming in Sikkim for increased productivity, nutrient uptake and diseases management	Dr. Laxuman Sharma	M.Sc., ICAR- NET Ph.D. thesis submitted & viva-awaiting

Progress Brief (*to be filled for each H-RA in separate row*):

RA No.	Research Objective(s)	Addressed Deliverables	Achievements	Research/ Experimental Work*
1.	• Develop the safe organic vegetable cultivation models for the region in 2 villages by using the microbial inoculums.	Microbial inoculums for organic farming in Sikkim.	At present we have isolated 30 isolates from rhizospheric soil of Pea plant (*Pisum sativum* L.) from different places from Sikkim. Based on the colony morphology on agar plate our isolates showed distinct morphologies. The some beneficial soil microbes secrete secondary metabolites such as	

	• Produce quality and safe organic vegetables and other crops without damaging environment in Sikkim. • Develop the technology to utilizes the natural resources and improves soil health in Sikkim. • Increase the productivity of vegetable and rice production in the region		enzymes and harmones which are potent for plant growth and diseases management. Therefore we also check whether our isolates produce metabolites such as Indole Acetic Acid (IAA) and siderophores. Interestingly few of our isolates showed Indole Acetic Acid (IAA) positive, similarly majority of our isolates showed siderophore positive based on the presumptive test. We also check our siderophores positive isolates for quantative estimination by CAS-Shuttle assay. Based on these assay we found two isolates PP2 (332.5%) and DP (507.5%) showed highest siderophore producer than other positive isolates.	

*Experimental work giving full details (in separate sheet, within 300 words) of experimental set up, methods adopted, data collected supported by necessary table, charts, diagrams & photographs. *Note*: Data, table and figures may be attached as separate source file (.docx, .xls, jpg, .jpeg, .png, .shp, etc.).

3. Fellowship Description at H-JRF Level

Himalayan Junior Research Project Fellows (H-JRFs)

H-JRFs Profile Description:

S. No.	Name of JRF	Date of Joining	Name of the PI	Qualification
1.	Divya Rai	19.06.2018	Dr. Laxuman Sharma	M.Sc., ICAR- NET
2.	Bahadur Singh Bamaniya	19.06.2018	Dr. Niladri Bag	M.Sc., ICAR- NET
3.	Kabita Gurung	02.01.2020	Dr. Niladri Bag	M.Sc., ICAR- NET

Progress Brief (*to be filled for each JRF in separate row*):

JRF No.	Research Objectives	Deliverable	Achievements	Research/ Experimental Work*
1.	• To characterize diverse genotypes on morphological and molecular basis. • To study the variability in the existing genotypes. • To establish inter relationship among various attributes under study	Screening of Sikkim *Houttuynia cordata* Thunb. germplasm for phenotypic (morphological) and genotypic (molecular) variability	To fulfill the above given objective permit for plant collection was applied in the month of June and was approved dated-06/07/2018. Tour for survey and plant sample collection was started from July 7th 2018, survey and collection of plant samples were done from four district of Sikkim. Locality of plant was examined, different	

	• To undergo GCMS of the selected germplasm		altitudes were recorded and collection of plant was done from east, west, north and south district of Sikkim. Total 65 sample with its altitudes were collected. To procure the altitudes “Garmin GPS map 78s”, were used, provided by Sikkim university, Gangtok. The survey and the collection of samples was done till 1st week of September, 2018. Collected plants were maintained in the nursery at Lingding village, Gangtok, East Sikkim. Intercultural operation such as weeding, irrigation, earthing up was done when needed. Soil test of field before planting wasdonefor organic carbon and NPK “Soil testing laboratory” Govt. of Sikkim,and pH was tested at the laboratory of Sikkim university. In the month of September, Extraction of fresh sample of leaves and roots for essential oil was performedbut have failed to give good result. Essential oil extraction through Clevenger is under process. On the month of February, 2019rhizome of the nursery plant were planted at three different localities with different altitudes (Lingding, Singtam and Yangyang). It was done to observe its morphological effects and now is in 2-3 leaf stage. Sample preparation for biochemical test and soil testing from different location is under process. On the month of March, 2019 test of carbohydrate	

			was performed which has shown the positive results in *Houttuyniacordata* Thunb. Meanwhile quantification of protein ofgeneral sample of Houttuynia was performed in spectrophotometer which has shown positive results and for which sample preparation is also under process.	
2.	• Characterization and analysis of physio- chemical properties of soil. • Study of culturable microflora from rhizosphere at the time of sowing and harvesting of crops. • To study polyphasic characterization of isolated microflora. • Characterization of phylogenetically relevant genes from isolated DNA from soil. • Correlation between types of microbial consortia in rhizosphere, growth and productivity as a result selection of compactable improved variety	Isolates will be screened for PGPR traits and quality enhancement of crops grown in Sikkim	Morphological characterization of soil isolates were completed in three different media (Nutrient Agar, Luria bertani agar, Actinomycetes agar) were used to isolate the general bacteria available in the soil at the time of sowing of crop. A total of 50 isolates were isolated from the soil at the time of crop sowing. On the basis of colony morphology and microscopy majority of the isolates were differ in each other. Further biochemical and molecular characterization of the isolates are under process.	
3.	• Survey to document the severity of disease in horticultural crops in the region. • To identify the botanicals on the basis of IK to be used to control the disease. • Isolation and identification of the causal	Document the severity of disease in horticultural crops in the region through survey and to identify botanicals on the basis of indigenous knowledge to be used to control the diseases	Till date tour to West and South district of Sikkim for survey has been conducted. The study was undertaken in selected Twenty four villages of West and South district of Sikkim. Methods employed in this study were designed with the purpose of providing baseline information on	

	organism of a disease in the region. • Control of disease using botanicals and other formulations (will be identified using IK of locals during survey).		use of plants for the preparation of bio-pesticides in local system through surveys, Participatory Rural Appraisals (PRA) and field visits to various areas of West and South Sikkim. The data were collected through structured and semi-structured interviews with native farmers. A total of 150 respondents were interviewed. After that few respondents were chosen to finalize status, utilization aspects, methods of preparation of local biopesticides/botanicals and to identify the most valuable plant species from farmer's perspectives. Thus, only specific and reliable information cross checked with informants is incorporated in the present study.	

*<u>*Experimental work*</u> giving full details (in separate sheet, within 300 words) of experimental set up, methods adopted, data collected supported by necessary table, charts, diagrams & photographs. *Note*: Data, table and figures may be attached as separate source file (.docx, .xls, jpg, .jpeg, .png, .shp, etc.).

4. Fellowship Description at Institutional/ University Level

Annual Deliverables/ Outputs (during the reporting year)

S. No.	*Deliverables/ Parameters*	*No.*	*Description*
1.	No. of Research Publications (monograph/ articles/ peer-reviewed articles):	Nil	[*Enclose file for description – max. 250 words*]
2.	No. of Data Sets generated:	Many	[*Enclose file for description – max. 250 words*]
3.	No. of Conferences/ Workshops attended:	8	[*Enclose file for description – max. 250 words*]
4.	No. of Sites/ Study Area Covered:	Whole Sikkim, i.e., i.e., East, West, North, and South Sikkim.	[*Enclose file for description – max. 250 words*]
5.	No. of Best Practices suitable for IHR:	NA	[*Enclose file for description – max. 250 words*]
6.	New Observations/ Innovations	NA	[*Enclose file for description – max. 250 words*]

5. Fellowship Concluding Remarks/ Annual Summary

Conclusions summarizing the achievements and indication of remaining work (within 300 words):

Bacterial isolates isolated from Pea rhizosphere and bulk soil samples from different villages of West Sikkim were identified based on morphology, biochemical and 16s rDNA sequencing with nr/nt database of NCBI. Isolates *Bacillus sp.* ARA showed phosphate solubilizing activity, similarly, *Bacilllus aryabhattain* PSB2 showed N2 fixation and siderophore production, *Burkholderiace nocepacia* SRD exhibited bio-control potential, *Kosakoniaoryzendophytica*YMA7 showed phosphate solubilizing activity and *Pseudomonas fluorescens* SRB showed Potassium solubilizing activity. Similarly, *Bacillus cereus* P4, *Paenibacillus nitroguajiacolicus* PP3, *Rhizobium etli* P18 showed good PGPR trait and one unidentified DC1 isolate showing good antifungal activity against Anthracnose causing fungus *Colletotrichum gloeosporiodes* YSI isolated from rice of Sikkim and this fungus cause blight diseases in rice and large cardamom in Sikkim. Selection bacterial strains for the preparation of inoculums and field trail is in progress and will be completed shortly.

In the other study regarding screening of *Houttuynia cordata* Thunb. germplasm found in Sikkim for phenotypic (morphological) and genotypic (molecular) variability survey and plant sample collection was in four district of Sikkim. Total 65 samples with its altitudes were collected. For further study collected plants are now maintained in the nursery at Lingding village, Gangtok, East Sikkim. Extraction of fresh sample of leaves and roots for essential oil are in progress. Sample preparation for biochemical test and soil testing from different location is also under process.

Under the other study towards development of rhizosphere microbial competency for yield and quality traits in selected vegetables for selecting improved line for organic farming, morphological characterization

of soil isolates completed. In this study three different media (Nutrient Agar, Luria bertani agar, Actinomycetes agar) were used to isolate the common bacteria available in the soil at the time of sowing of crops in different parts of Sikkim.

A total of 50 isolates have been isolated from soil samples collected from various parts of Sikkim at the time of crop sowing. On the basis of colony morphology and microscopy majority of the isolates were differ in each other.

The collected bacterial species is under the investigation for their proper identification. One pseudomonas species have been identified as plant pathogenic bacteria and after thorough study, the species will be firstly reported from the organic farming of Sikkim. The completed study may communicated in the reputed journal after their virulence study.

1. Screening of bacteria for PGP traits
    a. Phytohormone production (IAA, GA3, ACC-Deaminase)
    b. Mineral Solubilization (Phosphorous, Zinc, Iron etc.)
    c. Antagonistic activity against pathogensofSolanaceae family.
2. Consortium Preparation and In-vitro evaluation
    a. Culture compatibility test.
    b. Pot Culture with the prepared consortium

For documentation and validation of indigenous knowledge based agronomic practice, microbial inoculants for growth promotion and diseases management in organic farming in the State Sikkim, till date tour to West and South district of Sikkim for survey has been conducted. The study was undertaken in selected Twenty four villages of West and South district of Sikkim. Methods employed in this study were designed with the purpose of providing baseline information on use of plants for the preparation of bio-pesticides in local system through surveys, Participatory Rural Appraisals (PRA) and field visits to various areas of West and South Sikkim. The data were collected through structured and semi-structured interviews with native farmers. A total of 150 respondents were interviewed. After that few respondents were chosen to finalize status, utilization aspects, methods of preparation of local biopesticides/botanicals and to identify the most valuable plant species from farmer's perspectives. Thus, only specific and reliable information cross checked with informants is incorporated in the present study.

On the basis of the survey work the plant species involved in the making of various formulations has been collected and the herbarium of the species is prepared. The plant species has been stored and the formulation has been prepared. The in vitro microbial assay of the plant extract are still under testing. After the in vitro assay gets completed the in vivo condition is yet to be performed.

The documentation part is over but the validation of the ITK based formulation is still to be covered in the coming session.

6. Specific Research Question(s) Addressed with Succinct Answer(s)

S. No.	*Research Questions Addressed*	*Succinct Answers (within 150-200 words)*
1.	NA	

7. Any Other Information

- In this study it is expected several beneficial microbes will be isolates from different vegetables rhizospheric soil and rice rhizospheric soil from different parts of Sikkim. Quantative extermination of Indole Acetic Acid (IAA), Gibberellic acid (GA) will be carried out. Identification of isolates by phenotypic and genotypic method and field trail of selected microbial inoculants and product validation will be established.
- Morphological, molecular and biochemical characterize different genotypes of *Houttuynia cordata* Thunb. will be established.
- Culture dependent microbial analysis, bacterial dynamics at sowing and harvesting period will be established which may improve overall productivity in organic farming.

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Report (hard copy) should be submitted to:

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National Mission on Himalayan Studies (NMHS)
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G.B. Pant National Institute of Himalayan Environment and Sustainable Development (GBPNIHESD)
Kosi-Katarmal, Almora 263643, Uttarakhand

Report (soft copy) should be submitted to:

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