

MINOR PROJECTS UNDERTAKEN BY 6th SEMESTER – 2018-19, BOTANY HONOURS
STUDENTS OF BOTANY DEPARTMENT; LADY KEANE COLLEGE, SHILLONG.

Submitted to:
Botany department
Lady Keane College


2019

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Bacteriological test of tamarind juice used in Panipuri and Alu muri from street food vendors in and around Shillong City

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Introduction

Street foods are prepared on the street or prepared from home by the vendor and sold on the street. Street vended foods are appreciated for their unique flavours and provide a source of affordable nutrients to the majority of the people especially the low-income group in the developing countries (Muzaffar *et al.* 2009). However in most cases, street foods are unhealthy and are frequently associated with diarrhoeal diseases due to unhygienic preparation and serving practices (Barro *et al.* 2006). Microbial contamination of ready-to-eat foods sold by street vendors and hawkers has become a major health problem. Street food vendors are mostly uninformed of good hygiene practices (GHP) and causes of diarrhoeal diseases (Mensah *et al.* 2002), which can increase the risk of street food contamination (Bhaskar *et al.* 2004; Tambekar *et al.* 2009). From the initial contamination of raw foods with pathogenic bacteria to subsequent contamination by vendors during preparation; there are many factors that should be considered for analyzing the hazards due to street foods (Mankee *et al.* 2003; Dawson and Canet 1991). The vendors can be carriers of pathogens like *E. coli*, *Salmonella*, *Shigella*, *Campylobacter* and *S. aureus* who eventually transfer these food borne hazards to consumers. Street vended chats like Panipuri, bhelpuri, alu muri, etc sold in almost all the cities throughout India and are consumed by huge population. In Shillong too various kinds of street foods are available which include panipuri, alumuri, alu chana, chow, momo, etc sold in different parts of the city. The present study aims to analyze the bacteriological contaminations of street vended food viz., Panipuri and alu muri from different parts of Shillong, Meghalaya, India.

Objective: To determine the bacterial contamination of tamarind juice used in Panipuri and alu muri by culture characteristics and gram staining.

Materials and methods

Sample collection

The tamarind juice used by the vendors for the preparation of Panipuri and alu muri were collected in sterilised sampling bottles and brought to the laboratory for analysis. Six samples

were collected from different sites in and around Shillong city viz. mawprem (M), Police Bazar (PB1 and PB2), Malki (MAL) and Laitumkbrah (L1 and L2).

Sample analysis

Presumptive test

For analysis 1 mL of the sample was inoculated in 5 mL MacConkey broth and incubated for 12-16 h. The microbial growth was observed as turbidity in the broth.

Confirmed Test

This test is used to confirm the presence of coliforms in water samples showing positive or doubtful presumptive test. In the confirmed test, the samples from the presumptive MacConkey broth tubes are streaked on to a selective differential medium for coliform. The medium commonly used is eosin-methylene blue (EMB) that is selective in nature because of the presence of the dye methylene blue which inhibits the growth of gram positive bacteria, allowing the growth of gram negative bacteria. EMB is differential in nature in that lactose fermenting bacteria gives coloured colonies (a positive confirmed test) due to the formation of complex in EMB that precipitates out onto the coliform colonies. Non-lactose fermenters produce colourless colonies on EMB agar.

Complete Test

Complete test is used as a confirmatory test for the presence of *E.coli* in the water sample. In this test, lactose positive colonies from EMB agar are inoculated into a lactose broth tube and streaked on a nutrient agar plate to perform gram staining. If there is production of acid and gas in the inoculation lactose broth and there are rod shaped bacteria showing gram negative reaction, this confirms the presence of *E.coli* in the water sample and is considered a positive complete test.

Identification of Coliform Bacteria by Gram Staining

Using a sterile technique, a smear of bacteria growing in a nutrient agar medium was prepared, dried and heat fixed. The smear was then flooded with crystal violet, allowed to stand for one minute and washed in a tap water. The smear was then flooded with gram's iodine mordant and dried for one minute and washed in tap water. 95% of ethyl alcohol was poured drop by drop into the smear until the crystal violet decolorized from the smear. The smear was again washed in water and then counterstained with safranin for 45 seconds. The smear was washed in tap water and dried with blotting paper. It was then examine under the microscope.

Media composition

1. Mac-Conkey broth: 35 of the powder dissolve in 1L distilled water and sterilize.
2. Eosin Methylene Agar (pH 7.2)
 - a. Peptone - 3.0g
 - b. Lactose - 1.5g
 - c. Sucrose - 1.5g
 - d. Di-potassium phosphate - 0.6g
 - e. Agar - 4.6g
 - f. Eosin yellow - 0.12g
 - g. Methylene blue - 0.0195g
 - h. Distilled water - 300ml
3. Lactose fermentation broth (pH-6.9)
 - a. Lactose - 5.0g
 - b. Peptone -5.0g
 - c. Beef extract - 3.0g
 - d. Distilled water - 1000ml
 - e. Bromocresol purple 2.0ml
4. Nutrient Agar:
 - a. Beef extract- 3g
 - b. Peptone- 5g
 - c. Agar- 15g
 - d. Distilled water -1000ml

Results

When the samples were inoculated in MacConkey broth, no turbidity was observed in sample collected from Mawprem. In the rest of the samples, viz, those collected from Police Bazar (PB1 and PB2), Laitumkhrah (L1 and L2) and Malki (MAL) turbidity in the MacConkey broth was observed (Table 1). When inoculated in Eosin-methylene blue (EMB) medium by streaking, all the plates showed bacterial growth but the sample M showed fewer colonies (Table 1). Further in Lactose fermentation broth, sample M showed no gas production but all the samples showed bacterial colonies in Nutrient Agar medium (Table 1). On performing gram staining, except sample M all other samples showed the presence of gram negative bacteria (Table 1). The study

thus revealed that out of the six samples analysed, five were contaminated with *E.Coli*. It can be concluded that the sample collected from Mawprem (M) is free from *E.Coli* while the rest are contaminated.

Discussion

Food hawkers in India are generally unaware of food regulations and have no training in food-related matters. They also lack supportive services such as water supply of adequate quality and rubbish disposal systems, which hamper their ability to provide safe food (Titarmare *et al.* 2009). Several authors have observed that bacteria from water and other sources constitute a risk for contamination during food vending (Bhaskar *et al.* 2004; Mosupye *et al.* 2000). Defective personal hygiene can facilitate the transmission of these pathogenic bacteria found in environment and on people's hands via food to humans (Tambekar *et al.* 2008; Mensah *et al.* 2002). Bacterial pathogens like *E. coli*, *S. aureus*, *Bacillus cereus*, *Salmonella*, *Shigella* are responsible for the food borne and diarrheal diseases. *E. coli* and other coliform bacteria could be due to inadequate hand washing by food workers and the absence of clean water (Chauhan *et al.*, 2015). A study in Amravati, India, 93% of panipuri water samples had high loads of bacterial pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella spp.*, *Pseudomonas spp.* and yeast (Tambekar *et al.*, 2011). The present study revealed the bacteriological characteristics of the samples collected only once from different sites in and around Shillong City. To get a clear picture, analyses have to be done regularly and throughout the year. Moreover, an elaborate study on bacteriological contamination of the tamarind juice can be done by biochemical tests and by using Cysteine Lactose Electrolyte Deficient agar (CLFD) medium where suspected colonies can be identified based on their morphological, physiological and biochemical features using microscopic observation, standard biochemical methods and cultural characteristics on CLFD such as yellow colored colonies of lactose fermenting *E.coli*, greenish blue or blue colonies of *Ps. aeruginosa*, mucoidal yellow to whitish blue colonies of *Klebsiella spp.* and deep opaque colonies of *S. aureus* (Hi-Media manual 2003). It is suggested that regular monitoring of the quality of street foods must be practiced to avoid any food-borne infection.

Table 1. Culture characteristics and gram staining

Sl No.	Sample	Observation				
		MacConkey broth	Eosin-Methylene Blue (EMB) medium	Lactose fermentation broth	Nutrient Agar medium	Gram staining
1	M	No turbidity	Few bacterial colonies	No production of gas	bacterial colonies	Gram positive
2	PB1	Turbidity	bacterial colonies	Production of gas	bacterial colonies	Gram negative
3	PB2	Turbidity	bacterial colonies	Production of gas	bacterial colonies	Gram negative
4	MA1	Turbidity	bacterial Colonies	Production of gas	bacterial Colonies	Gram negative
5	L1	Turbidity	bacterial colonies	Production of gas	bacterial colonies	Gram negative
6	L2	Turbidity	bacterial colonies	Production of gas	bacterial colonies	Gram negative

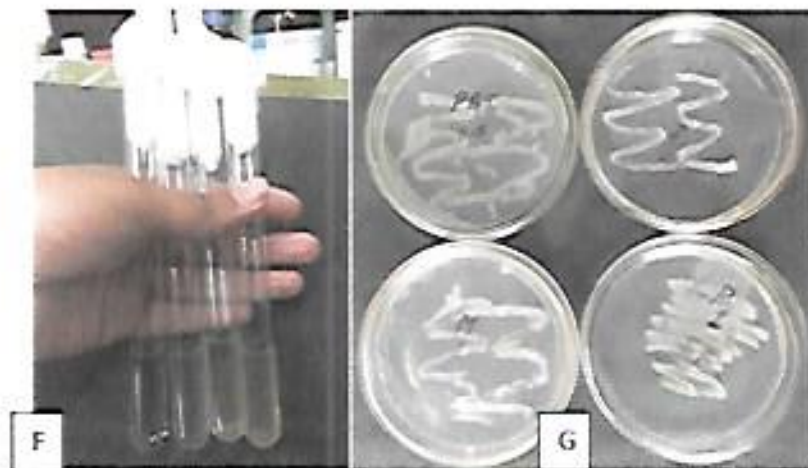
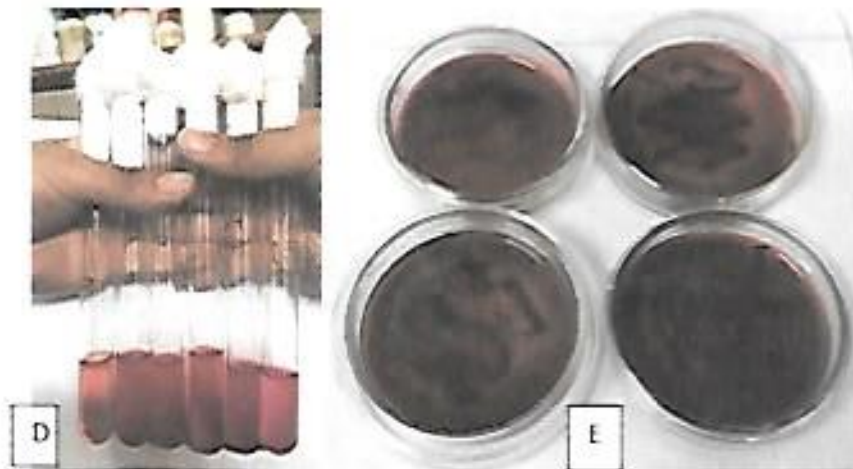


Fig: A-C: inoculation of bacteria, D: MacConkey broth inoculated with samples, E: bacterial colonies in eosin-methylene blue (EMB) medium, F: lactose broth with bacterial culture, G: bacterial colonies in nutrient agar medium.

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Asymbiotic seed germination of *Phalaenopsismannii* Rchb.f

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INTRODUCTION

The ever increasing rate of deforestation in the state of Meghalaya for commercial, mining, industrial establishment in the last few decades has led to a drastic declining trend in the population of endemic and rare plant. Destruction of the natural forest habitats inevitably threatened biological diversity of the area, particularly orchids (Sinuet *et al.* 2011). Majority of orchids are epiphytic by nature, and are associated with forest trees, hence clearing of forest for commercial and agricultural purposes have a direct impact on the population of these sensitive plants family. This local decline in orchid diversity and abundance is being repeated in many protected areas throughout the state.

Moreover, commercial exploitation of orchids for ornamental purpose has considerably depleted their natural population in the wild. Presently, all the orchids have been included under Appendix-II of CITES. To avoid this serious threat, attempts should be made to multiply the orchid so as to prevent them from extinction.

In orchids the embryo is few-celled at the time of seed maturation and its proper development takes place only during germination of seeds (Senthilkumar, 2001). Moreover, as the orchid seeds do not have sufficient reserve food materials to take care of the growth of embryo during germination (Richardson *et al.*, 1992). These seeds have to depend on some external source for nutrients so as to enable their undifferentiated embryo develop into a protocorm. Mycorrhizal association with particular fungal species is necessary for early stages of development (Rao, 1977). The *in vitro* seed germination has an added advantage to overcome this reproductive barrier as well as for the conservation of the orchid species. So far *in vitro* seed germination has been achieved in many orchid species (Sharma and Tandon, 1987; Kumaria and Tandon, 1991; Alamet *et al.*, 2002; Lo *et al.*, 2004; Buyan *et al.*, 2004; Luan *et al.*, 2006). However, the reports depict that the responses of *in vitro* seed germination of orchids varies from species to species and a thorough study on each and every species of orchid is needed.

Phalaenopsis orchid, belonging to the Orchidaceae family, is one of the most important orchids and most popular epiphytic monopodial orchid, which is grown for commercial production of cut flowers and potted plants. It is known for its beautiful flowers in terms of large size, form, color and known to be originated from the island of Borneo (Gnasekaran *et al.*, 2010).

The epiphytic orchids i.e., *Phalaenopsismannii*, is chosen for the present study as there is no specific protocol for the asymbiotic seed germination of the above mentioned orchid.

Phalaenopsismannii orchid, also called as Mann's Phalaenopsis (named after a German orchid enthusiast Gustav Mann). This species was described by Reichenbach in 1871. Mann's Phalaenopsis is native to the eastern Indian Himalayas, Assam, Nepal, Bhutan, Sikkim, Myanmar, southern China and Vietnam.



Phalaenopsismannii

MATERIALS AND METHODS

Eleven to twelve months old capsules of *P. mannii* were harvested from the plants and washed thoroughly under running tap water. The capsules were surface sterilized by dipping them in 70% alcohol for about 3 minutes followed by washing with distilled water. This process was repeated 2-3 times. The sterilized capsules were split aseptically open and powdery seeds were scooped out and inoculated on the surface of agar gelled nutrient medium.



Innoculation Chamber

Two nutrient medium Vacin and Went (VW) (1949), White (1943) supplemented with 6-benzylaminopurine (BAP) and 2,4-dichlorophenoxyacetic acid (2,4-D), either individually or in combinations were used for seed germination. The media were solidified with 0.8% (w/v) agar and the pH of the media was adjusted to 5.8 ± 0.02 prior to autoclaving at 121°C for 15 minutes. The cultures were incubated at $25 \pm 2^{\circ}\text{C}$ for 12h photoperiod with cool white fluorescent tube.

Once the spherules were formed, observations were recorded at an interval of one week to trace different stages of development of protocorms.

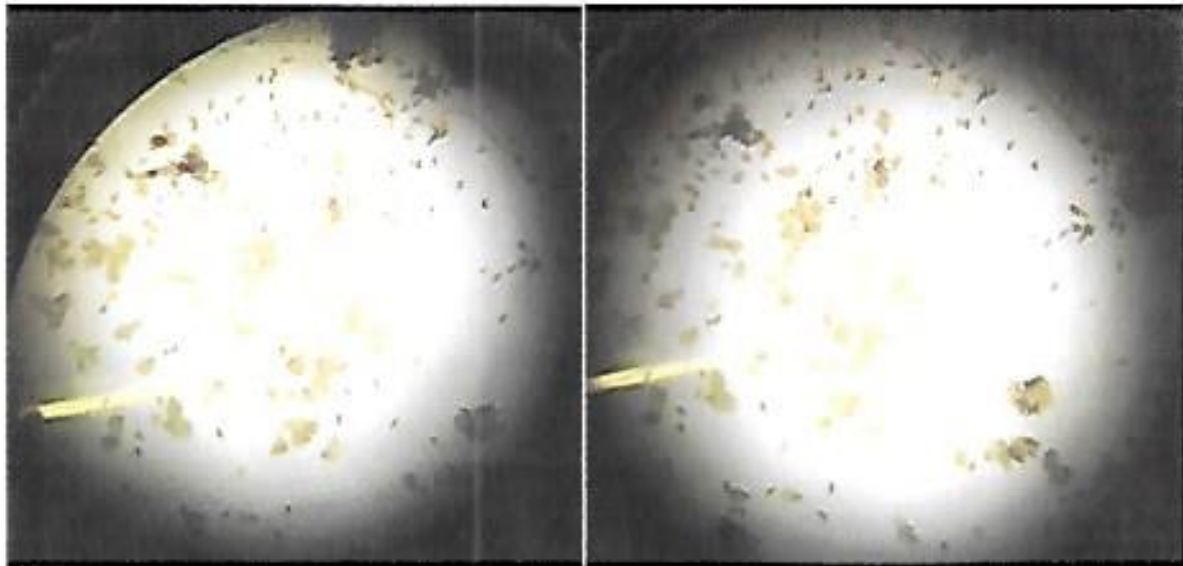
Table 1. Germination of seeds of *P. mannii* on agar solidified different media.

Medium	PGRs (mg/l)		Spherule formation	Protocorm formation	Germination %
	BAP	2,4-D			
VW	-	-	-	-	*
	0.5	-	+	-	-
	1.0	-	+	-	-
	-	0.5	+	-	*
	-	1.0	+	-	*
	0.5	0.5	+	-	-
	1.0	1.0	-	-	*
White	-	-	-	-	*
	0.5	-	-	-	*
	1.0	-	-	-	*
	-	0.5	-	-	*
	-	1.0	-	-	*
	0.5	0.5	-	-	*
	1.0	1.0	-	-	*

* - Contaminated

- - No Germination

+ - Very less number of spherule formation



Spherule formation

RESULTS AND DISCUSSION

The immature embryos in young seeds procured from the green undehisced capsules showed some positive responses in the nutrient media supplemented with growth regulators but the frequency and onset of germination response varied with the nature of growth stimulus. The choice of medium and conditions of growth significantly affected the germination of orchid seeds.

In the present study on *P. mannii*, formation of spherules (spherical structures) was observed in VW medium supplemented with Plant Growth Regulators (PGRs) but further germination was not observed in all the medium studied, this must be due to physical factors and not of nutrient supply. Various workers who had work on asymbiotic seed germination on *Phalaenopsis* had observed good responses with VW medium (Utami and Hariyanto, 2019; Abbaszadehet *al.*, 2018; Shekarrizet *al.*, 2014).

No spherules or germination was observed in White medium with or without PGRs and this must be due to nutrient as well as physical factors. There had been no report on the used of White medium in asymbiotic seed germination of *Phalaenopsis* orchid.

The success of *in vitro* orchid seed germination and seedling growth is not only restricted to nutrient supply but also to physical factors like temperature and light which greatly influence the physiology and development of orchid seedlings (Arditti and Ernst, 1984).

CONCLUSION

Since orchid seeds respond differently to various nutrient medium during germination, therefore further investigation with other medium should be carried out.

Preliminary analysis of drinking water quality of few restaurants in Shillong, East Khasi Hills, Meghalaya

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Introduction:

Assessment of drinking water quality should be regularly and constantly monitored so as to ensure the various drinking water sources are safe for human consumption. The current preliminary assessment aimed at achieving a few of these concerns on the quality of water.

Objectives:

- a. To ensure the water we drink is safe
- b. To create awareness on the important of maintaining quality drinking water.

Methods:

Sampling: four restaurants were selected randomly and the drinking water is collected after the students explain to the owners about their concerns.

Waters collected are analysis for microbial contamination: Presumptive test using MacConkey broth medium; Confirm test using cosin-methylene blue (EMB) broth medium.

Analysis of Physical and chemical quality composition of drinking water using Aquasol water analysing kits

Result:

Two water samples (LS & M) show no microbial contamination while microbial contamination was confirmed in two water samples (IN and PK).

Dissolve iron in the four water samples ranges from 0.1ppm (LS, PK & IN) to 0.5ppm (M), Chloride ranges from 20ppm to 50ppm, free chlorine is 0.1ppm in all water samples, fluoride is 0.2ppm in all samples. Nitrate ranges from 1.0ppm to 2.5 ppm and alkalinity ranges from 40ppm to 70ppm. pH level of three water samples (LS, M, IN) were found to be slightly acidic (5.5-6.0) while one water sample (pH 8.0) is slightly alkaline. Turbidity ranges from 5NTU to 20NTU.

Table 1: Bacterial test of drinking water

Water sources	Presumptives Test	Confirmed Test
LS	-ve	No contamination
PK	l ve	Contaminated with <i>E. coli</i>
M	-ve	No contamination
IN	l ve	Contaminated with <i>E. coli</i>

Table 2: Preliminary physical and chemical analysis of water

Water sources	Iron (ppm)	Chlorine (ppm)	Free chlorine	Fluoride (ppm)	Nitrate (ppm)	Hardness (ppm CaCO ₃)	Alkalinity (ppm)	pH	Turbidity
LS	0.1	20	0.1	0.2	1.0	300	40	5.5	20 NTU
PK	0.1	40	0.1	0.2	1.0	150	60	8.0	5 NTU
M	0.5	40	0.1	0.2	1.0	200	40	6.0	10 NTU
IN	0.1	50	0.1	0.2	2.5	200	70	6.0	20 NTU

Conclusion and Discussion:

Two water samples (IN and PK) show positive bacterial test and was confirmed using FMB broth medium while two water samples (LS & M) show no microbial contamination at all. Presence of bacteria of drinking water is an important indicator of the quality of water.

Dissolve iron, Chloride, free chlorine, fluoride, nitrate, and alkalinity fall within the permissible limits (Table 2). pH level of three water samples (LS, M, IN) were found to be slightly acidic while one water sample (PK) is alkaline. Turbidity of two water samples (PK and M) fall within the permissible limits while two water samples (LS and IN) are on the slightly higher side.

Project Title: Preliminary Phytochemical Screening of wild edible plants of East Khasi Hills District, Meghalaya.

Students: Baniakmen Kshiar, Baiahun Kharkongor, Riakor lin Hoojon, Onisha Soh, Ibarilang Pathaw and Iairikmen Warjri. VI Semester, BSc, Botany Honours students.

Teacher incharge: K. H. M. Pala

Introduction

The Indian subcontinent represents one of the richest diverse genetic resources. Of the estimated 250,000 species of flowering plants at global level, about 3,000 are regarded as food source, in which only 200 species have been domesticated. Global diversity in vegetable crops is estimated at about 400 species, with about 80 species of major and minor vegetables reported to have originated in India. However, with the advent of cut-and-burn agriculture and green revolution/ commercialized agriculture, the development project areas and related activities of these diverse resources are declining at a fast pace. Overgrazing, deforestation, and over exploitation of native resources under range situations have eroded the biodiversity from this unique ecosystem. Moreover, traditional knowledge about these important indigenous plant species has also decreased in the younger generation influenced by urbanization. Indigenous plant species provide a variety of products like food, medicines and raw materials. The Indian subcontinent has been one of the rich emporia of 2,500 plant species used in indigenous treatment and food sources.

Vegetables play an important role in human diet. They are our main source of vitamins, minerals, fiber, essential oils and phytonutrients, besides containing low amounts of fat and calories¹.

Beside their nutritive properties, they also exhibit medicinal and physiological potentials. Consumption of fresh vegetable enables full assimilation of vitamins on the human body². The basic elements in leafy vegetables provide alkalizing effects, neutralizing the acidity caused by other foods of animal origin³. Certain vegetables are highly beneficial in the treatment of various diseases. Spinach is beneficial in the treatment of kidney troubles. Lettuce can be used as a food remedy for insomnia. Water leaf has been also implicated medically in the management of cardiovascular diseases like stroke, obesity, and so on³. The "E.firin" (scented leaf) serves as a decongestant for head, colds, bronchitis and sinusitis.

The amounts of the nutrient constituents in the commonly used leaf vegetable species in Meghalaya have been studied to some extent, the lesser known regional and local species remain virtually neglected. Lack of information on the specific nutrients and phytochemicals in a large number of the native vegetables species with which Meghalaya is richly endowed is partly responsible for their under exploitation especially in areas beyond the traditional localities where they are found and consumed.

Objectives:

This paper aims to screen the phytochemicals content of the wild edible plants available in the northeast India, Meghalaya, and in turn hope to highlight their important as viable food source which may be use in alleviating hunger, malnutrition and improving health, thereby making a difference in livelihoods. Further research may wish to concentrate on the anti-microbial properties of these valuable vegetables commonly found in the northeast India, Meghalaya.

Sample collection:

The wild edible plants selected are as follows:

1. *Centilla asiatica*: *Centilla asiatica*, commonly known as centella, locally known as Kynbat moina (Khasi), *Brahmi* (Garo) or Asiatic pennywort. It is an herbaceous, perennial plant, belonging to the family Apiaceae. It is native to the wetlands of Asia. It is used as a culinary vegetable and as a medicinal plant.
2. *Diplazium esculentum*: This plant is a fern and locally known as Jhur Tyrkhang (Khasi) It is edible and found throughout Asia and Oceania. They may have mild amounts of toxin but no major toxic effects are recorded. It is used as vegetable and salad.
3. *Eryngium foetidum*: It is a tropical perennial herb belonging to the family Apiaceae. It is native to Mexico and South America. It is widely used in seasoning and marinating. It is locally known as Etucha-bellock (Garo).
4. *Houtunia cordata*: It is also known as fish mint or fish leaf and locally known as jamyrdoh (Khasi and Jaintia). It is native to Japan, Korea, South China and Southeast Asia. The leaves and roots are consumed as vegetables, the leaves and flowers are also used in making tea.
5. *Fagopyrum esculentum*: It is locally known as Jarain (Khasi and Jaintia) and is used as a vegetable.
6. *Oenanthe crocata*: It is locally known as Jatira. It is an indigenous plant of Meghalaya used as a vegetable and salad.
7. *Begonia spp*: Locally known as 'Jajew' (Khasi). Begonia is a genus of perennial flowering plant belonging to the family Begoniaceae. The genus contains more than 1,800 plant species. The species are native to moist subtropical and tropical climate in South and central America, Africa and southern Asia. It is consumed as vegetables, cooked or prepared as chutneys.
8. *Ficus*: Collectively known as Fig Trees and locally as 'Diengsohphymai' (Khasi). It is a genus of 850 species belonging to family Moraceae. The leaves are consumed as vegetables.
9. *Curcuma*: Locally known as 'Khawiang' (Khasi). Curcuma is a genus of 100 accepted species belong to the family Zingiberaceae that contains such species as turmeric. The stem is grinded and added in rice and vegetables preparation for its aroma.
10. *Begonia spp*: Locally known as 'Jalyngdop' or 'Jajew'. Mostly cooked as vegetables with dried fish.

11. *Gynura*: Locally known as 'Jali' (Khasi) belonging to family Asteraceae. Mostly consumes as vegetables and salad.
12. *Amblanthus* locally known as *DIENGSOIISHIER* its young leaves is mostly cooked with dried fish and consumed as vegetables.

Fresh wild edible plants were procured from the local market and Pynursla, East Khasi Hills, Meghalaya. The plants were identified using relevant floras. Blemish-free leaves of the plants were brought to the department, washed to remove all debris stored in the deep freezer at -20°C for further analysis.

Material and Methods:

Preparation of plant extract:

100 g of freeze dried leaf samples the wild plants were grinded in 100 ml of methanol. The resultant mixtures were then filtered with Whatman No. 1 filter paper. The filtrates were collected in containers and stored at 4°C until further use.

Methods

The extracts were subjected to qualitative analysis for the presence of different phytochemical constituents by following the standard methods ^{4,5}.

Test for alkaloids

Wagner's and Dragendorff's tests

2 mL extract was mixed with 2 mL of 1% aqueous HCl, taken into two separate test tubes and 6 drops of Wagner's and Dragendorff's reagents were added. The formation of a reddish brown precipitate with Wagner's reagent and orange-red precipitate with Dragendorff's reagent indicated the presence of alkaloids.

Test for saponins

1 mL extract was shaken vigorously with 20 mL of distilled water in a graduated cylinder for 15 minutes. Persistence of froth indicated the presence of saponins.

Test for cardiac glycosides

Keller-Killiani's test

2 mL extract was treated with 1 mL of glacial acetic acid containing few drops of FeCl_3 solution. To this, 2 mL of concentrated H_2SO_4 was poured from the side carefully. The formation of reddish brown at the junction of two layers and the formation of bluish green at upper layer indicated the presence of deoxysugar and hence cardiac glycosides.

Tests for steroids

Salkowski's test

2 mL of the extract was taken in a test tube and then 2 mL of chloroform and 2 mL of concentrated sulphuric acid were added to it and shaken well. Chloroform layer appearing red and acid layer showing greenish yellow fluorescence was considered as an indication for the presence of steroids.

Test for anthraquinones

Modified Borntrager's test

About 0.5 g of the extract was taken in a dry test tube and 5 mL chloroform was added and shaken for 5 min. The extract was filtered, and the filtrate was shaken with an equal volume of 100% ammonia solution. A pink, violet or red colour in the ammoniacal layer (lower layer) indicated the presence of anthraquinones.

Test for coumarins

3 mL of 10% NaOH was added to 2 mL of extract and formation of yellow color indicated the presence of coumarins.

Test for phenols

Ferric chloride test

5 mL of extract was allowed to react with 1 mL of 5% ferric chloride solution. Greenish black coloration indicated the presence of phenols.

Test for tannins

Gelatin test

1% gelatin solution containing 10% sodium chloride solution was added to the extract. Formation of white precipitate indicated the presence of tannins.

Test for flavonoids

Shinoda's test

To the test solution, a small piece of magnesium ribbon was added followed by drop wise addition of concentrated hydrochloric acid. Pink-tomato red color indicated the presence of flavonoids.

Test for carbohydrates

Molisch's test

Fehling's Test . 2 mL of the extract was added in 1 mL of a mixture of equal volumes of Fehling's solutions A and B, and was boiled for few minutes. Red precipitate formation was an indication for the presence of carbohydrates (reducing sugars).

In this test the presence of aldehydes but not ketones is detected by reduction of the deep blue solution of copper(II) to a red precipitate of insoluble copper oxide. The test is commonly used for reducing sugars but is known to be NOT specific for aldehydes. For example, fructose gives a positive test with Fehling's solution as does acetoin.

Two solutions are required:

Fehling's "A" uses 7 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ dissolved in distilled water containing 2 drops of dilute sulfuric acid.

Fehling's "B" uses 35g of potassium tartrate and 12g of NaOH in 100 ml of distilled water.

These two solutions should be stoppered and stored until needed.

Iodine test for starch

The crude extract was mixed with 2 ml. of **iodine solution**. A dark blue or purple coloration indicated the presence of starch (carbohydrate).

Dissolve potassium iodide in about 200 cm³ distilled water and then add iodine crystals. Make the solution up to 1 litre with distilled water. It is essential to prepare it 24 hours before it is required, as iodine is slow to dissolve.

Test for anthocyanins

2 ml. of extract was added to 2 ml. of 2N HCl and ammonia. The appearance of blue-violet color indicated the presence of anthocyanins.

Test for proteins

Ninhydrin test

Few drops of Ninhydrin reagent were added to the extract. Appearance of blue color indicated presence of amino acid.

Millon's test

Crude extract when mixed with 2 ml. of Millon's reagent, white precipitate appeared which turned red upon gentle heating that indicated the presence of protein.

Test for phlobatannins

The crude extract of sample was boiled with 2% HCl. The deposition of a red precipitate was taken as evidence for the presence of phlobatannins.

Test for lignin

2 ml. of 2% (w/v) furfuraldehyde was added to the test solution. Formation of red color indicated the presence of lignin.

Table 1: Plants procured from Local market, East Khasi Hills, Shillong, Meghalaya.

	<i>Centella a</i>	<i>Diplazium m</i>	<i>Oenanthe esculenta</i>	<i>Oenanthe ereceta</i>	<i>Houttu cordata</i>	<i>Eryngiu m foetid</i>	<i>Fagopyru m esculenta</i>	<i>Begonia sp. 'Jajow'</i>	<i>Ficus sp.</i>	<i>Cureta ma sp.</i>	<i>Begonia sp. 'Jalyngdo p'</i>	<i>Cynur a sp.</i>	<i>Ambyanthus sp.</i>
Saponins	+	+	+	+	+	-	+	+	+	+	-	+	-
Cardiac glycosides	+	+	+	+	+	+	+	+	X	-	+	+	+
Steroids	-	-	-	-	+	-	+	+	+	X	-	-	-
Anthraquinone s	-	-	-	-	-	-	-	+	-	-	-	-	-
Phenols	-	-	+	+	+	-	+	-	+	-	-	-	+
Tannins	-	-	+	-	-	-	-	-	+	-	-	-	-
Flavonoids	-	-	-	-	-	-	-	+	+	+	+	+	-
Protein	+	+	+	+	+	+	+	X	-	-	-	-	-
Carbohydrate	+	+	+	+	+	+	+	-	X	-	-	+	-
Anthocyanins	-	-	-	-	-	-	-	-	-	X	-	-	-
Starch	-	-	-	-	-	-	-	-	-	-	-	-	-
Phlobatanins	-	-	-	-	-	-	-	-	+	+	+	+	-
Coumarins	-	-	-	-	-	-	-	+	+	+	+	+	+
Lignin	-	-	-	+	-	-	-	-	-	+	+	X	-
No. of phytochemicals present	4	5	6	6	6	3	6	7	9	7	5	5	3

Present (-), Absent (-), Traces (X)

Results:

Saponins was found present in almost all of the plants studied except *Curcuma* and Cardiac glycosides was found present in all except *Eryngium foetidum*, *Begonia spp* (Jalyngdop) and *Amblyanthus as* as shown in Table 1. Anthraquinones, Starch and Anthocyanins were found to be absent in almost all of the species studied except traces of Anthocyanin were found to be present in curcuma species and presence of Anthraquinones was observed only in *Begonia spp* (Jajew). *Begonia spp* (Jajew), *Ficus* and *Curcuma* are the three species of plant studied having the highest number of phytochemicals present, i.e. 7,9,7 respectively.

Conclusion and Discussion:

The study revealed that *Begonia spp* (Jajew), *Ficus* and *Curcuma* species had the highest number of phytochemicals present, whereas the least was observed in *Eryngium foetidum* and *Amblyanthus*. The rest of the plant species studied showed the presence of at least 50% metabolites present. The various phytochemical constituents suggest that the plants can be a good source of food, natural antioxidants and their consumption should be encouraged when available. Hence, further biological studies of these wild edible plants are needed to explore the beneficial effect in human health.

Phytochemical analysis of the wild edible plants showed the presence of a number of medicinally active secondary metabolites (Table 1). Plant cells produce two types of metabolites. Primary metabolites viz. carbohydrates, lipids and proteins, which are involved directly in growth and metabolism. Carbohydrates, fats and proteins are important nutrients of life. The plants rich in carbohydrate content provide more energy. The key role of carbohydrate in the body is to supply energy and is responsible for doing various activities in our daily life^{6,7}. Most natural products are compounds derived from primary metabolites such as carbohydrates, fatty acids and amino acids and are generally known as secondary metabolites. Secondary metabolites viz. alkaloids, saponins, phenolics, flavonoids, tannins, essential oils, terpenes, steroids, lignin etc. are considered as the products of primary metabolites. These secondary metabolites are not involved in metabolic activities and are the main sources of pharmaceuticals, food additives, fragrances and pesticides^{8,9}. Fruits and vegetables constitute an important source of bioactive compounds which differ widely in terms of structure, biological properties, and mechanisms of actions. Various phytochemical constituents of plants are known to be responsible for antioxidant, antimicrobial, anti-larvicidal, and anti-inflammatory activities¹⁰. Steroids have analgesic, antibacterial and anti-inflammatory properties¹¹. Saponins show anti-inflammatory and hemolytic activities. They have bitterness and cholesterol binding properties^{12,13}. Steroids and saponins are responsible for the activities of central nervous system¹⁴. Glycosides have anti-diarrhoeal activity¹⁵ and can reduce blood pressure¹². Fruits and vegetables are good sources of phenolics, flavonoids, and anthocyanins which are responsible for antioxidant, anti-carcinogenic and health-promoting properties^{16, 17}. Flavonoids, a group of phenolic compounds, are free radical scavengers which prevent oxidative cell damage through their water soluble property and also possess strong anti-cancer activity⁵. Tannins are used for the treatment of diarrhoea and

dysentery, and have received immense attention in many fields due to their physiological activities like antioxidant, antimicrobial and anti-inflammatory^{5, 17}. Coumarins act as potential antioxidants with the ability of scavenging free radicals and chelating metal ions⁵.

Acknowledgement

Authors are grateful to the Lady Keane College, Shillong, for financial assistance and to faculties of the Department of Botany, Lady Keane College, for identification of most of the wild edible plants and to the Head, Department of Botany, Lady Keane College for providing laboratory facilities and guidance.

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Population studies of *Sonerila squarrosa* Wall.

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¹Lady Keane College, Shillong - 793001, Meghalaya; *email:klchaudhary31@gmail.com

Abstract

The present paper reports the effects of anthropogenic disturbance in *Sonerila squarrosa* Wall. (Melastomaceae) populations from East Khasi Hills, Meghalaya. The study showed that the plants are negatively affected by anthropogenic disturbance; with changes in individual shoot height, weight, colony size and number of reproductive shoots.

Introduction

Sonerila Roxb. is a genus of the family Melastomaceae (>870 spp in 47 genera; Michelangeli *et al* 2013). *Sonerila* are plants of low herbaceous or semi-woody shrub habit primarily distributed in tropical Asia (Clausing & Renner, 2001). It includes creeping or erect and occasionally acaulescent forms.

Sonerila squarrosa Wall. Was first described by Nathaniel Wallich (Flora Indica, 1820). It was last collected by J.D. Hooker & T. Thomson from Khasi Hills in 1859, after which no collections have been made. This plant has been re-discovered from Khasi Hills recently by KL Chaudhary *et al.*. The plant grows in only limited areas on large boulders within the riverine habitat, resulting in its rarity. These riverine habitats are frequented by people for fishing, picnics and tourism who climb on to the boulders where these plants grow either for stream crossing or photography and cause damage through physical contact and trampling while traversing through. Thus, a study of effects of disturbance on the population behavior and reproduction is important for the conservation of the plant.

New plant locations

The plant previously been collected from four locations in the Sohra area. During the current year's fieldwork, additional locations have been discovered in different streams of the Sohra area. The newly discovered sites are located in Pung-pung stream in Mawlyndiar village and in a stream running through the Kharmih Varieties Spot, a picnic spot enroute to Dainthlen. Population within the Kharmih Varieties Spot stream is very small and restricted to just one spot on a large inaccessible boulder while the Mawlyndiar village stream has a higher population.

Population studies

The studies focused on the effect of anthropogenic disturbance on the plants. Those populations that are located in the direct paths of travel, are small, patchy with very few shoots per colony while those that are generally in inaccessible locations are bigger, with more shoots and more reproductive output.

The entire population of these sites were counted, measured and categorized: as 'disturbed' if they grow at places which are frequented by humans, these are characterized by small-sized shoots, small colony size and very little fruiting, 'semi-disturbed' if they grow at places which are occasionally frequented by humans; these are characterized by slightly bigger shoots, and bigger colony size and more fruiting as compared to disturbed ones, 'undisturbed' if they grow at places which are very

rarely frequented by humans as they are located outside the paths frequented by humans or are in inaccessible places, these have significantly bigger shoots and bigger colony size and fruiting as compared to others. Three sites with significant population were chosen for detailed study; however study at one site remained incomplete due to inclement weather and high water flow in the stream.

Results

The new population discovered at Mawlyndiar and the earlier discovered population have been studied and the data is tabulated (Table 1 & Fig 2).

Table 1.

Location	Av Shoot Ht (Cm)	Shoot Ht Of Reproductive Shoots (Cm)	Shoot Ht Of Unreproductive Shoots (Cm)	Av No. Of Shoots/ Reproductive Colony	Av. No. Of Shoots/ Unreproductive Colony	Av No. Of Fruits/ Colony
Laitmawsiang Disturbed	2.05	3.08	2.11	2.79	3.43	1
Laitmawsiang Semi-Disturbed	3.19	4.53	2.99	11.57	6.55	2.06
Laitmawsiang Undisturbed	3.68	5.37	2.94	13.13	7.75	5.25
Mawlyndiar, Disturbed	3.12	4.08	4.08	6.33	5.50	1.44
Mawlyndiar Semi-Disturbed	3.75	5.30	5.30	6.67	7.00	2.58
Mawlyndiar Undisturbed	4.25	6.23	6.23	14.86	8.00	4.2

As can be seen, the disturbed sites have smaller heights, number of shoots per colony, and lower fruiting compared to undisturbed colonies. The differences in biomass between these are also significant (Table 2 & Figure 3).

Figure 1: *Sonerila squarrosa* in its habitat



A disturbed *Sonerila squarrosa* colony



A semi-disturbed *Sonerila squarrosa* colony



An undisturbed *Sonerila squarrosa* colony



A disturbed *Sonerila squarrosa* colony at Mawlyndiar



An undisturbed *Sonerila squarrosa* colony at Mawlyndiar



A rare case of fruit with germinated seeds of *Sonerila squarrosa* colony at Mawlyndiar

Table 2.

Location	av fresh wt, plant	av lf fresh wt, plant	av dry wt, plant	av dry wt, lf
Laitmawsiang disturbed	0.91	0.33	0.41	0.13
Laitmawsiang semi-disturbed	2.45	1.22	1.56	0.64
Laitmawsiang undisturbed	8.41	3.77	2.53	0.70
Mawlyndiar undisturbed	16.04	8.48	6.23	1.64

Figure 2. Average shoot heights, number of shoots and fruits per colony

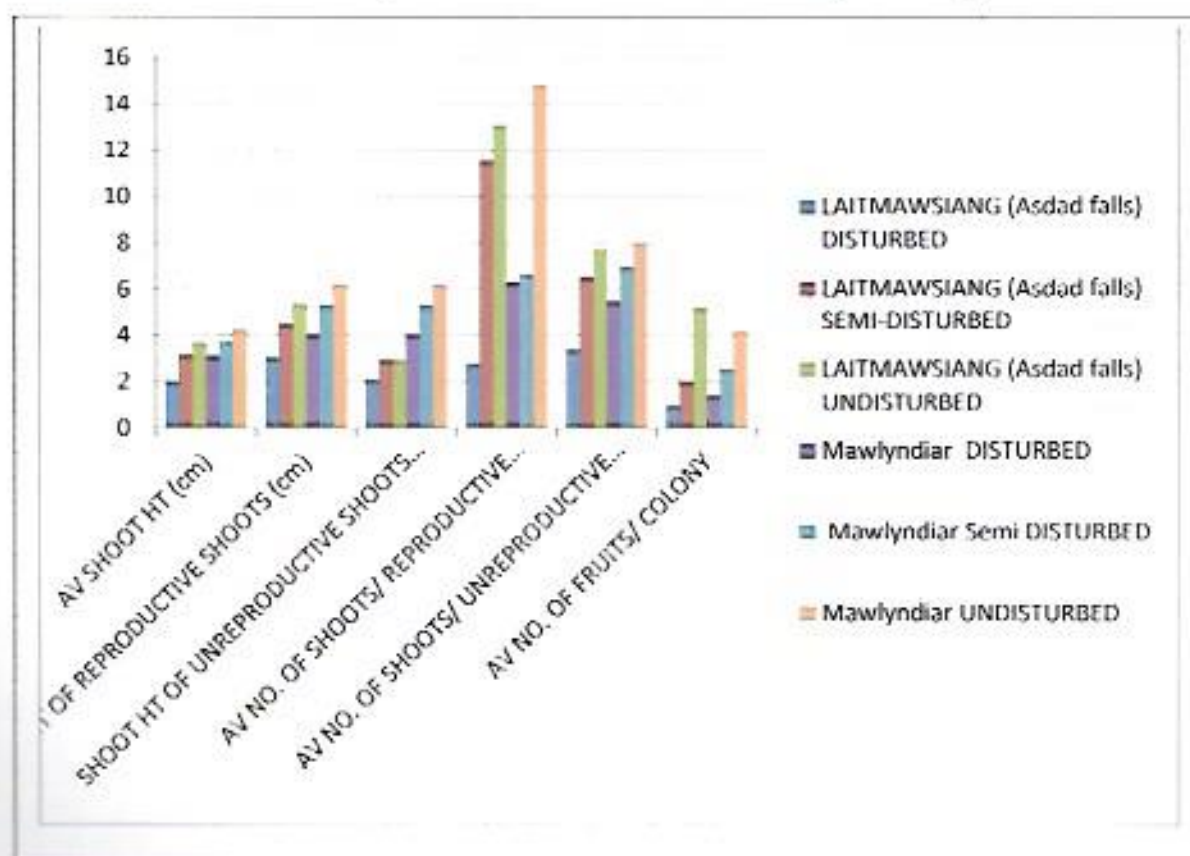
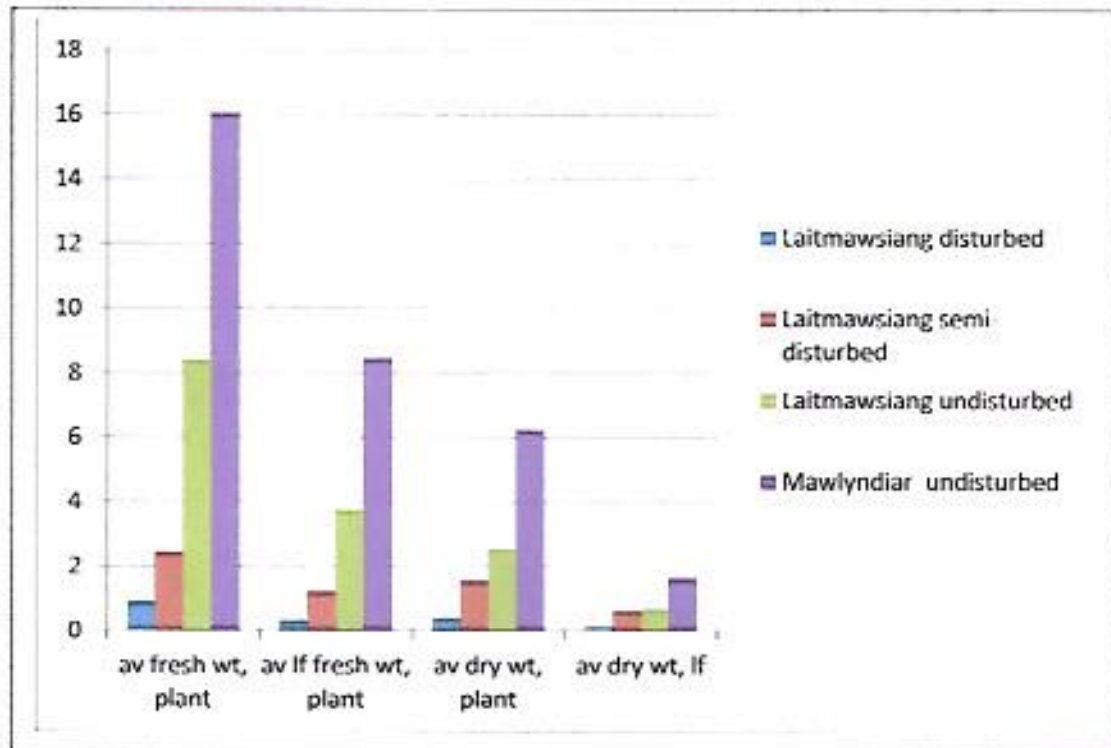


Figure 3: Fresh and dry shoot and leaf biomass at different sites



Threats

The threats to the population are many, the most important being its small and restricted population size. The plants being clonal also spread vegetatively but at exceedingly slow rates. Upon fragmentation of the colony, the fragments if separated from its substratum do not have a chance of re-establishment due to the slippery nature of the substratum and are more likely to be lost. Therefore, new populations at currently uninhabited locations can arise only through sexual reproduction.

Result

The disturbed populations show a significant decrease in growth and reproductive capacity as a result of disturbance. These populations are threatened with site-specific extinction due to loss of reproductive capacity-both vegetative as well as sexual.

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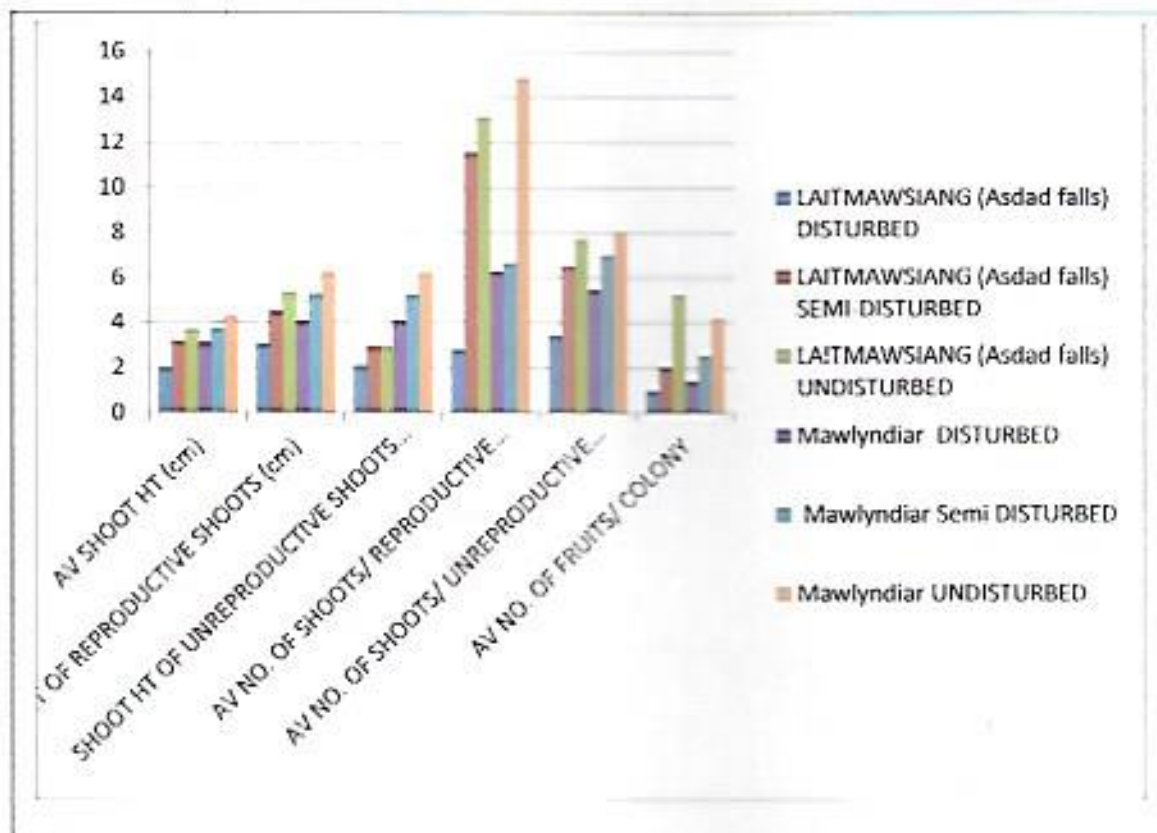
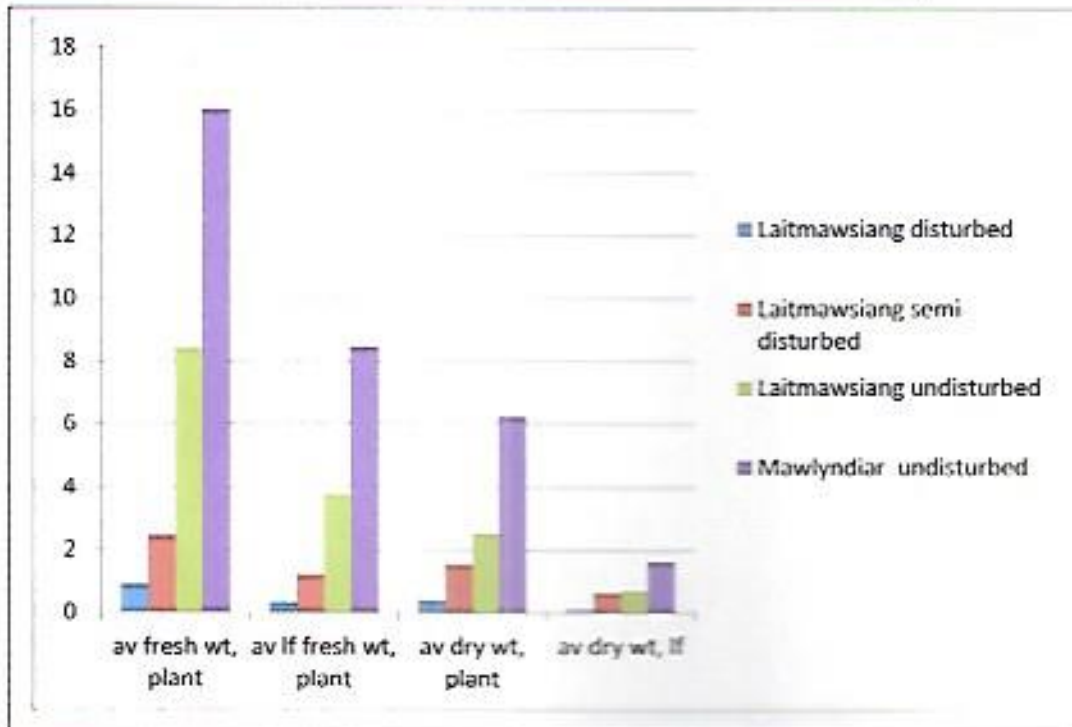


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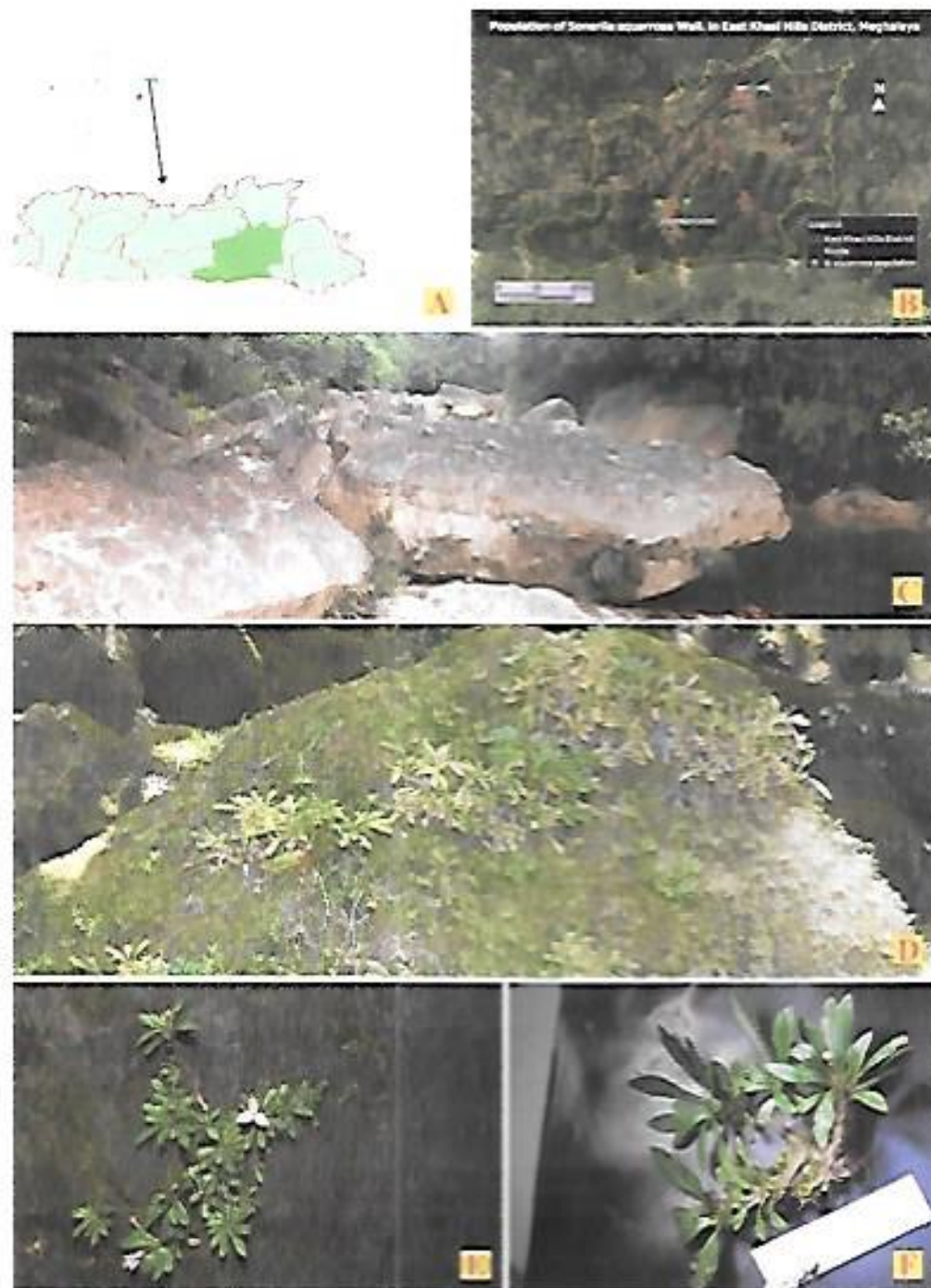


Fig. 1: A-Location of East Khasi Hills B-Satellite map of East Khasi Hills C-Habitat D-*Somerila squarrosa* growing on boulder in stream bed E-A single plant showing ramets and flowers F-A single plant



Fig. 2: A-A *S. squarrosa* colony with flowers. B-A flower. C-A leaf and flower dissected to show the different parts. D-Transverse section through ovary. E-Fruits (capsules). F-Seeds.

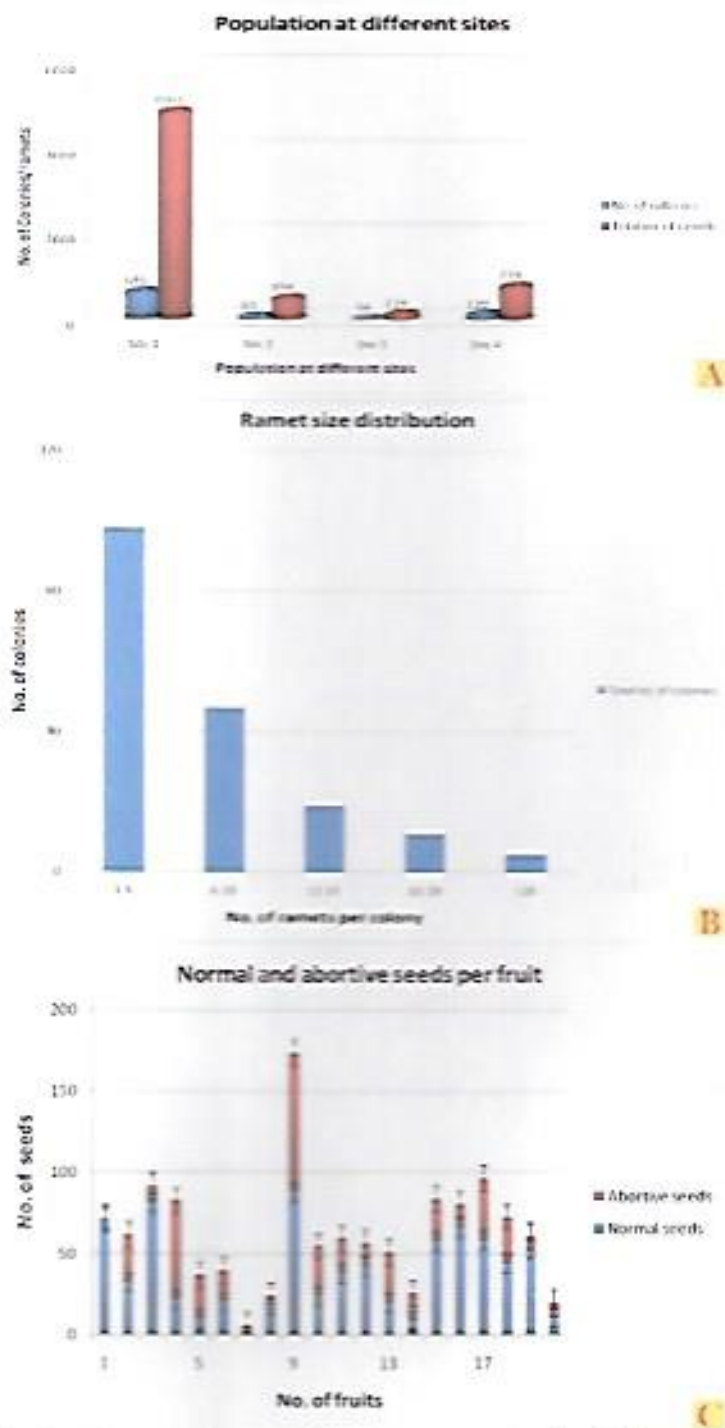


Fig. 3: Graph showing the populations at various sites **A**-Number of individuals and ramets at various sites. **B**-Size distribution of colonies (number of ramets per colony). **C**-Number of normal and abortive seeds per fruit. (\pm S.E.)



ALAN WANKHAR

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FASHION DESIGNER

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Dated, Shillong, The 19th April, 2018

To
The Vice Principal
Lady Keane College,
Shillong

Ref : Your letter No. C/4B/2003/COC/110/122 dated 16.03.2018

Subject : Completion of Internship Programme.

Dear Madam,

This is to inform you that the following students of Fashion Designing of your Institution, have successfully completed their Internship Programme under my guidance for the period from 19th March to 19th April 2018.

During the period of the programme, they have proved to be sincere, hard working, eager to learn more and also helpful.

The above mentioned students are :-

1. Ai Talin Marbaniang
2. Alacrity Bareh
3. Pleasureful Kshiar
4. Ibaphyllashisha Nongkseh
5. Rosa Mystica Wahiang
6. Kerda Rangad
7. Irealty Langstieh
8. Baladiang Rumnong
9. Roselyne Nongbri
10. Ribhalin Sanglyne
11. Kynjai Thabah

I wish them all success in life.

Thanking you.

Copy to: FD teachers
: Dr. A. Nongstang


Mr. Alan Wankhar

25-04-18
103

Report on field trip to Sohra

On the 6th December 2018, the history department, Lady Keane College undertook a field trip to Sohra as part of its going project on "Historical Sites in Sohra". The students of the fifth Semester of the department accompanied by Dr. Mrs. Merryville Jyrwa started from the college around 7:30am. It may be noted that British relations with the Khasi Hills being a part of the curriculum and with the administration of the Khasi Hills, Cherrapunji became the seat of the Government where the political agent to the Governor General was stationed and it was made the capital of Assam in 1832 and a Sanatorium as well. Hence, Cherrapunji provides a corpus of information relating to British relations with the hills. It may be added that remnants of British structures and edifices survive till today, thereby making Sohra an ideal site for students to research upon. It may be stated that an initiative was taken by former sub-divisional officer of Sohra civil sub-division, Brahmadev Ram Tawari, who sent a proposal to the state government to write to UNESCO to declare the area a World Heritage site. The advent of the Welsh Missionaries in the Khasi Jaintia Hills is a subject that evoked the interest of the students, The Presbyterian Church of Wales sent the first missionary to Khasi Hills, Rev Thomas Jones and his wife, Anne. They travelled from Wales to Calcutta and from there they landed in Saitsohpen, Sohra on 22nd June 1841 which was the head quarter of the then Sylhet Light Infantry. Remnants of their missionary works can still be seen in Sohra, hence it became more apt for the department to take up this project.

The old Cherra or Sohrarim was the original village, hence the first stop was Sohrarim. The students thronged the villagers to enquire about the old houses where they learned to have existed for decades, some were as old as 150-160 years. On reaching Sohra, the group first went to Ramakrishna mission started in 1924. It is not only a seat of learning but its contribution to the welfare of the Spciety is remarkable. Te next stop was the Nongsawlia Presbyterian Church and the Cherra Teachers Training Centre. The first Church building was built upon a hillock of the present Nongsawlia Cemetery but the building was destroyed by the great earthquake in June 1897. To mark the 175 Anniversary of the coming of the Gospel, the Khasi-Jaintia Presbyterian Assembly celebrated its 175 Jubilee Celebration at Nongsawlia Presbyterian Church. The present church was built in 1898. One can also see the remnants of the White Bungalow at Saitsohpen where Thomas Jones was provided shelter by Lieutenant William Lewin. The other prominent site visited was the Thomas Jones School of Mission which was established in 1886. This building represented early 20th Century colonial architecture in Sohra and claims to be one of the oldest major buildings which survived the great earthquake. It is also known as the Cherra Theological College.

The students were elated to discover some of the graves of the British including that of Thomas Jones and his wife besides others who laid to rest in the Nongsawlia Church Cemetery.

Almost all of the graves had an epitaph on them but only few had withstood the ravages of time. One such was that of J. Duncan. This was followed by a trip to the monument of David Scott which has been erected at Saitsohpen in remembrance of David Scott Agent to the governor General North East Frontier of Bengal.

After lunch the group proceeded from Saitsohpen to Kut Madan area of Sohra. On the way the students with the help of the local people were able to locate the remains of the Bungalow of Harry Inglis, a local merchant who monopolized the lime and orange trade in the 1830s and 1840s. The group further proceeded to the erstwhile Shadwell Estate in Kut Madan which was in the 1860s known as "Emmaville", the home of John Bird Shadwell Assistant Commissioner of the Khasi and Jaintia Hills and his wife Emma. In the Estate, one notices the burial grounds of the late Shadwell family. After his death, John Shadwell married into the local Langstieh clan.

Another noteworthy visit is the Circuit House Sohra which was established in 1830 and is the oldest building in Sohra. It is documented that Pandit Jawaharlal Nehru visited Cherrapunji in 1952 and a tree was planted by his daughter, Indira Gandhi, in front of the building. A monument commemorating the spread of the Brahmo Samaj in 1889 was also located in Sohra. The students were also taken to the Kcep Thang Brier or the Burning Ghat of the Syiems of Cherrapunji. A large number of memorials were discovered which were yet to be researched upon.

The students were fascinated by the deep sense of history that Sohra possessed. Our next task is to try to locate the railway track of the Cherra Companyganj Sate Railway which was said to have opened for traffic in 1887. It was said that passengers and goods were ferried to and fro between Tharia, a small hamlet in Cherrapunji and Companyganj now in Bangladesh. Its main objective was to connect Sylhet and Cherrapunji. However it was getting late, this would entail travelling to Tharia and Mawsmat. The students were reluctant to leave. However, we ended our trip and returned back to Shillong exhausted but deeply aroused by a deep passion to continue researching on the various facets of Sohra's deep heritage and culture.

Submitted by,



(Dr. Mrs. Merryvella Jyrwa)

DEPARTMENT OF EDUCATION

LADY KEANE COLLEGE

Report of the Educational Survey

OBJECTIVE : - TO ASSESS THE INFRASTRUCTURAL CONTENT OF PRIMARY SCHOOLS UNDER MAWKYNREW BLOCK.

The VI Semester Students ,Department of Education , Lady Keane College conducted a survey on the 14th of March 2019 to assess the conditions of Infrastructure of Primary schools falling under Mawkynrew Block. The total number of students who undertook the survey was 28 They were divided into 5 different groups. The students interacted with the Head of the respective Institutions,the teachers and the students.A tool containing questions relating to the Infrastructure of Schools was used during the survey .

In assessing the Infrastructure of primary schools, certain criteria were set for evaluation of the above objective . These criteria include certain basic essentials facilities considered essential for any schools such as Electricity Connection,Drinking Water facilities,Staff room,Toilets,Playground,etc.

Under the survey conducted, five schools were selected for evaluation of our said objective,the schools selected were

1. Mawkynrew Presbyterian Upper Primary School
2. R.C. Upper Primary School, Pashang
3. R.C. Lower Primary School, Pashang
4. R.C. L.P and U.P School , Laitdiengsai
5. Jatah Nonglyer Prebyterian School

Based on the Survey which was conducted, it was found that the Average number of students in each school is 67,the type of school is Co Educational Most of the Primary Schools lacked basic facilities such as Principal's Room,Staff Room,Separate Toilets,School laboratory,etc

The Survey was conducted by the students of the Department under the guidance and supervision of the teachers of the department of Education

The duration taken for preparation and to conduct the Survey was 8 days (7th of March - 14th of March 2019).

The Principal Investigator of the Educational Survey that was conducted in Mawkynrew Block ,East Khasi Hills District ,Meghalaya is Mrs Kerdalari Dkhar,Assistant Professor,Department of education,Lady Keane College.


K. Dkhar

Faculty Member,Department of Education



LADY KEANE COLLEGE

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NAAC Re-Accredited: "A" Grade

Ref. No. C/28/2018/DF-Hu/462-B

Date : 11.07.2018

To,

Ms. J. Buam,

Head, Department of History,

Lady Keane College,

Shillong.

Dear Ms. Buam,


As approved by the Governing Body, this is to inform you that an amount of Rs. 28,000.00 (Rupees Twenty Eight Thousand) only is hereby sanctioned for your Departmental Project under the title "*Historical Sites in Cherrapunji*" for the year 2018-2019.

In this regard, I would like to request you to conduct the Programmes planned by your department within the 31st of March, 2019. In case, your department is unable to complete all programmes within the said date, re/new application shall be accepted for the next budget, i.e. 2019-2020 between February - March, 2019.

Learning outcomes of seminar/ workshop/talk/field trip conducted by your department for the year has to be submitted to IQAC.

Also, you are requested to give a brief write up in the College Magazine if seminar/ workshop/talk/field trip is organized/conducted.

Yours sincerely,


Principal
Lady Keane College,
Shillong.



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NAAC Re-Accredited: 'A' Grade

Ref. No. C/28/2012/DE-Edu/462-A

Date : 4.07.2018

To,

Mr. J. Pyngrope,

Head, Department of Education,

Lady Keane College,

Shillong.

Dear Mr. Pyngrope,

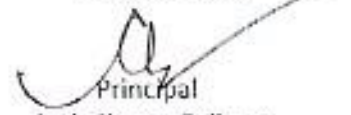
As approved by the Governing Body, this is to inform you that an amount of Rs. 15,000.00 (Rupees Fifteen Thousand) only is hereby sanctioned for your Departmental Project under the title "*To assess the infrastructural content of Primary Schools under Mawkynrew Block*" for the year 2018-2019.

In this regard, I would like to request you to conduct the Programmes planned by your department within the 31st of March, 2019. In case, your department is unable to complete all programmes within the said date, re/new application shall be accepted for the next budget, i.e. 2019-2020 between February – March, 2019.

Learning outcomes of seminar/ workshop/talk/field trip conducted by your department for the year has to be submitted to IQAC.

Also, you are requested to give a brief write up in the College Magazine if seminar/ workshop/talk/field trip is organized/conducted.

Yours sincerely,


Principal
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NAAC Re-Accredited: "A" Grade

Ref. No. C/29/2018/DE-Bot/S11-A

Date : 02.08.2018

From: Dr. (Mrs.) C. Massar,
Principal,
Lady Keane College,
Shillong.

To,
Mr. K.H.M. Pala
Head, Department of Botany,
Lady Keane College,
Shillong.

Sub: Sanction of Departmental Students' Projects.

Dear Pala,

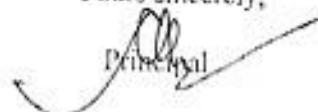
As approved by the Governing Body, this is to inform you that the amount of Rs. 35,000/- (Rupees thirty-five thousand) only is hereby sanctioned for the following Students' Projects of your Department for the year 2018-2019.

Sl. No.	Title of the Projects
1	Population studies of <i>Sonnerita squarrosa</i> in Sohra and adjoining areas
2	Bacteriological analysis of street vended foods in and around Shillong City
3	Phyto chemical screening of wild edible vegetables collected from Pymursla, East Khasi Hills, Meghalaya.
4	Asymbiotic seed germination of <i>Phalaenopsis manii</i>
5	Preliminary analysis of drinking water quality in Shillong

In this regard, I would like to request you to conduct the Projects planned by your department within the 31st of March, 2019. In case, your department is unable to complete all projects within the said date, re/new application shall be accepted for the next budget, i.e. 2019-2020 between February - March, 2019.

Report of the Projects conducted by your department for the year has to be submitted to IQAC.

Yours sincerely,


Principal