
Identification of landslide mitigative plant species and the rhizospheric fungal associations responsible for their incidence from landslide areas of East Sikkim

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The main objective of this research was to identify the landslide mitigative vegetation types (trees, shrubs and herbs) and understand their distribution and occurrence in the eight landslide zones of East Sikkim; Andheri, Amdogolai, Beto, Chandmari, Ganeshtok, Namchebong, Namli, Penlong, by making comparative studies of the plant vegetation forms in the other two landslide-free zones Bhusuk and Merung in East Sikkim and see if rhizospheric associations had an effect on their occurrence. Forty two efficient shrub species with excellent root shear strength to hold the soil mass during rainy season and dry seasons were identified. The shrub plant *Thysanolaena maxima* was found to be the most efficient plant species with a potential to bind the soil mass firmly and enhance the stability of land mass. It's association with rhizospheric Vesicular Arbuscular Mycorrhiza (VAM) was also studied to understand the occurrence and nature of VAM species associated with *Thysanolaena maxima*. *Glomus* sp. was found to be the dominant VAM species in the rhizosphere of *Thysanolaena maxima*. In addition, analyses of rDNA sequences revealed that uncultured *Ascomycota* species were found to be the dominant fungal species within rhizosphere of landslide mitigative shrub species *Thysanolaena maxima*. The information can be used for deriving the ecological conditions for the respective mitigative plant species, assessment, restoration, conservation and administration of natural vegetation in these areas.

Key words: Mitigation, genetic, rhizosphere, landslide, VAM, fungi, spores, bioengineering

INTRODUCTION

Plant vegetation plays a major role in controlling landslides, as it promotes soil stability by providing necessary soil bearing shear strength within the root system (Lepcha *et al.* 2010). Besides keeping the environment fresh, plant vegetation also plays a role in stabilizing the land ecosystem. The shear strength provided by root resist the external tension which possesses high erosive value (Lepcha *et al.* 2009). Tree roots have the ability to resist tension, thereby increasing the shear strength of shallow soils through mechanical reinforcement (Maffra *et al.* 2019).

One of the major factors for landslide generation is rainfall for which the canopy structure is another major comprehensive plant parameter that helps to protect the soil mass from direct bombardments of strong and heavy rain falls. During rainfall vegetation's leaf canopy intercepts rain and the root system act as an anchor and reinforces the

soil and provides stability to the land surface (Lepcha *et al.* 2009). The plants having good architecture of root system and canopy system can be considered as mitigative species that can hold and conserve the soil and thereby mitigate the landslides in the region.

Soil fungi are considered to be important natural biological agents for attaining the functions within the soil related to nutrient cycles, disease suppression and water dynamics. The soil fungal hyphae helps to absorb colonized nutrients from the roots. Being plastic in nature, fungi has an ability to survive in any adverse and unfavorable conditions breaking down organic matter and controlling the generation of carbon and nutrients (Zifcakova *et al.* 2016) thereby acting as natural decomposers (Yang *et al.* 2012). Vesicular Arbuscular Mycorrhiza (VAM) is an important natural microbial source for sustainable growth and development of plant species. Reports suggest that 80% of plant species forms mycorrhizas (Van der Heijden *et al.* 2015); Arbuscular-mycorrhizal fungi (AMF) plays a fundamental link between

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plant and soil (Ingraffia *et al.* 2019). The soil provides support to the plant, which is the primary producer. The quality of soil pertains not only towards the yields of agricultural productivities but also initiates appropriate management, a guarantee for its long term stability. Furthermore, VAM helps to minimize soil erosion by providing continuous vegetation cover. To form such cohesive impacts within soil mass, an intensive symbiotic association between the Vesicular Arbuscular Mycorrhiza (VAM) and their host plant and host soil is equally important. The mechanism performed by specific VAM organisms have reasonable impacts on the nature of soil property, affecting the fate and fertilities of land and its floral existence. Glomales (*Glomus* sp.), a fungi under *Zygomycetes*, promotes symbiotic association of VAM with the plant species, which colonizes the root of many plant species and exhibits a vital role in plant mineral nutrition.

This study identifies mitigative plant species amongsts shrub species which promotes strong soil binding capacity based on root soil shear strength. It also seeks to identify the rhizospheric and VAM fungal species associated with the mitigative plant species in the landslide prone areas and landslide free zone areas, which in turn will help to identify and understand the mechanism of exact soil fungi species responsible for sustenance of the mitigative plant species in the landslide prone zones and for creation of mitigative conditions. It additionally looks into the population ratios of vesicles and arbuscules in the specified mitigative species root system in order to understand the VAM species involved in colonization of these hosts as they have a profound effect ultimately on the incidence and occurrence of these mitigative vegetation in landslide free areas.

MATERIALS AND METHODS

Identification and selection of landslide and landslide free zones

Meticulous review of literature on landslides in East Sikkim was done from various information sources such as peer review journals, online web sources, news papers, magazines and physical visits. Eight landslide areas of East Sikkim namely Andheri Landslide, Beto Landslide (27°23'42.1"N, 88°38'22.0"E), Namchebong Landslide

(27°15'05.5"N, 88°35'11.5"E), Namli 9th Mile Landslide (27°16'54.7"N, 88°35'31.2"E) [Active Landslide zones] and Amdogolai Landslide (27°19'19.1"N, 88°36'26.8"E), Chandmari Landslide (27°20'19.2"N, 88°37'25.7"E), Ganeshtok Landslide (27°20'28.4"N, 88°37'16.7"E) and Penlong Landslide (27°22'34.3"N, 88°37'39.9"E), [Non-active Landslide zones] were selected for the study. Two landslide-free zones namely Merung zone (27°20'14.4"N, 88°39'03.8"E) and Bhusuk zone (27°21'00.7"N, 88°40'19.9"E) were selected to attain comprehensive comparative studies of Landslides. All selected sites (Fig.1) were thoroughly physically surveyed before undertaking the study.

GIS remote sensing

Application of GIS and Remote Sensing software was executed from State Remote Sensing Centre, Sikkim State Council of Science and Technology, Department of Science and Technology and Climate Change, Government of Sikkim. Arc GIS software, version 10.0 was used for image processing, registration, classification of raster images, in creating vector layer like shape file, Arc coverage etc. and was used to draw several digital images such as Landslide location map, Slope and Vegetation map.

Soil analysis for the determination of Erodibility 'K' factor

The soil testing was done in the Laboratory of Department of Tea Management, North Bengal University (NBU), Darjeeling, West Bengal.

The Physico-chemical parameters of the soils have been analyzed according to the method of Jackson (1973) and the erodibility factor (K), which helps to understand and categorized the risk status of landslides, were determined using following equation (Goldman *et al.* 1986).

Where f_p is particle size, P_{om} is percent organic matter, S_{struc} is soil structure index, f_{perm} is profile permeability class factor, and 1.292 is conversion factor from British units to metric units.

Plant Vegetation diversity

Field surveys were conducted to observe the actual plant diversity in the eight landslide areas of East

Sikkim, in which four landslide areas, Beto landslide, Namli landslide, Andheri landslide, and Namchebong landslide were active while four others such as Chandmari landslide, Ganeshtok landslide, Amdo golai landslide and Penlong landslide were inactive landslide areas. To do a comparative study, two non-landslide regions (virgin regions free from landslides), Bhusuk region and Merung region in east Sikkim were chosen

following formulas were implemented to determine the Relative Density, Frequency and Importance value of Plant Biodiversity of specific landslide region (Table 2).

$$K_{\text{fact}} = 1.292[2.1 \times 10^{-6} f_p^{14} (12 - P_{\text{om}}) + 0.0325(S_{\text{struc}} - 2) + 0.025(f_{\text{perm}} - 3)]$$

$$\text{Relative Density} = \frac{\text{Total No. of individuals of a species}}{\text{Total No. of individuals of all species}} \times 100$$

$$\text{Frequency} = \frac{\text{Total No. of quadrats in which species occur}}{\text{Total No. of quadrats studied}} \times 100$$

$$\text{Relative Frequency} = \frac{\text{Frequency}}{\text{Total Frequency}} \times 100$$

$$\text{Importance Value} = \text{Values of Relative Density} + \text{Value of relative dominance} + \text{Relative Frequency}$$

Bioengineering: Shrub Root reinforcement studies

Observing the morphology and architectures of all shrub plant species in all the landslide and landslide free zones, plant body measurement and calibration including height length, breadth length, root length, root, and shoot canopies were completed.

The pull out test methodology introduced by Tsukamoto (1987) and Abe and Iwamoto (1986), was implemented to study the root shear strength. By taking one species from each recorded herbs and shrubs species of landslide prone areas. Their root holding capacity was measured by using spring balance measurement meter. The plucking thread attached to the spring balance meter was dipped down to the bottom of the plant species and the entire plant root along with soil was pulled out manually.

The soil borne by the root was measured. The total height of plant, its shoot height, canopy breadth, root length, breadth were measured. The experiments were conducted during two different seasons; one in rainy seasons (July-September)

another in dry seasons (December-January) [Fig.6: A,B, Table. 2]. The study has helped to identify *Thysanolaena maxima* belonging to Poaceae family, as a durable and efficient mitigative shrub plant species, which has a potential to bind the soil mass and promote the land stability.

Rhizospheric soil spore analysis

The rhizospheric soil samples of mitigative shrub plant *Thysanolaena maxima* from all the research sites of landslide and landslide free zones were collected and taken to the laboratory for analysis. Spore extraction was done from the 100g of soil samples from all the ten landslide sites following wet sieving and decanting method (Gerdemann and Nicolson, 1963). 100 gms of soil sample was mixed with water in a beaker and left for a time till the material completely sedimented down to the bottom of the beaker. Only the supernatant (along with the surface floating material) was collected into a separate beaker and filtered. The filter paper was divided into eight sectors for ease of sampling and placed in funnel. The sieving process separated the water and spore material. The residual material on the filter paper was dried and examined under a phase contrast microscope (LEICA DM 1000) with photographic attachment. The spores were identified, photographed and quantified from each of the eight sections of funnel's filter paper by picking up spores from each section and placing them on to a slide.

Spore colonization percentage (%) was calibrated by means of following formula.

$$\text{The Spore Colonization (\%)} = \frac{\text{No. of spore segment}}{\text{Total no. of spore segments observed}} \times 100$$

Vesicular Arbuscular Mycorrhiza (VAM) spore analysis from root

The study of VAM was conducted from the root portion of the mitigative species *Thysanolaena maxima* of landslide areas. The root was collected and cut into 3 cm. Trypan blue staining of total mycelium was done; Roots were cleared in 10% (w/v) KOH for 15 min and autoclaved at 14 lb (pound pressure) for 20 min. The solution drained out and the roots were digested with 2% (v/v) HCl (Hydrochloric acid) for 30 seconds. Roots were then removed from HCl and washed with purified water/distilled water and bleached in alkaline H₂O₂

+ NH_4OH (Carbonium hydroxide) for 1 minute. (Alkaline H_2O_2 was made by adding 3 ml of NH_4OH to 30 ml of H_2O_2 and 567 ml of single distilled water). The bleached roots were then washed with distilled water and treated with 0.05% (w/v) Trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and water) for 15 minutes at 120°C . (0.05% = 50 mg in 100 ml of distilled water). The root segments were mounted on a slide with 50% (v/v) glycerol for destaining and viewed under the microscope. The prepared slides were viewed under a Phase-Contrast microscope (LEICA DM 1000) at the research laboratory of Microbiology Department, Indian Council of Agricultural Research (ICAR) Centre, Tadong, Gangtok and the VAM were quantified from every slide. The number of VAM were obtained along with its percentage (%). A total of 180 observations were made and the VAM images were archived.

VAM root colony was determined by means of following formula:

$$\text{VAM root Colonization (\%)} = \frac{\text{No. of Mycorrhizal root segment}}{\text{No. of root segments observed}} \times 100$$

Rhizospheric Fungal and DNA isolation, PCR and sequencing

Roots of the selected mitigative species colonized by fungi were washed with dH_2O and Chloroform, measured (1800 g) and poured liquid Nitrogen (N_2) and grind to power. Collected in centrifuge tube and added 1000 μl of pre-warmed 100 ml extraction buffer [20 g of CTAB 2%, 10 ml of 100 mM Tris (pH 8.0), 4 ml of 20 mM EDTA (pH 8.0), 28 ml of NaCl (1.4 M) dipped at 60°C for 30 minutes in water bath]. One volume i.e. 1000 μl of Chloroform-isoamylalcohol (24:1) was added to the sample, vortex/spin the sample and centrifuged at 4°C for 5 minutes at 5000 RPM. The supernatant was collected and to this equal volume of Chloroform-isoamylalcohol (24:1) was added to the sample; mixed well and centrifuged at 4°C for 5 minutes at 5000 RPM. The supernatant was transferred into fresh Eppendorf tube and 0.5 μl RNase A (2.5 μl RNase [10 mg ml^{-1}]) was added and incubated at 37°C for 30 minutes. One volume of Chloroform-isoamylalcohol (24:1), was added depending upon current volume of the sample after the above incubation process, if its 10 μl (RNase A+sample), then 10 μl Chloroform-isoamyl-alcohol (24:1) was added to it and the sample centrifuged at 4°C for 5 minutes at 5000 RPM. The supernatants were collected and two-

thirds volume of Isopropanol was added on it. The sample tube was inverted properly and incubated at 4°C for 15 minutes. After this the sample were centrifuged at 13000 RPM (10290 g) for 10 minutes at 4°C . The supernatant liquid was gently poured out leaving only the formed DNA pellet. The pellet was treated with 500 μl of 70% ethanol and centrifuged with 13000 RPM at 4°C for 5 minutes. The 70% ethanol was poured out and discarded leaving only the DNA pellet, which was air dried for 1 h and eluted in 1000 μl 1XTE Buffer (made up of 900 μl DH_2O and 100 μl of 10XTE) after dissolving of the sample.

Database statistics analysis

All data were statistically analyzed. The R based tool, Rstudio- was used to calibrate and exhibit all the graph images including boxplots.

RESULTS

GIS and Remote Sensing

DISCUSSION

Understanding and identifying mitigative plant species in landslide areas requires a holistic ecological approach and collation of inputs from different sources; live field surveys, application of Geographical Information System (GIS) software, satellite imagery maps of landslide zones, location and elevation map of landslide and landslide-free zones, forest/vegetation map of East Sikkim and aspect maps (Fig. 1, 2), vegetation characteristic as well as its distribution pattern and even soil composition and their microbial rhizosphere composition.

Soil in the forest ecosystem perform a key ecological function (Kiran and Kaur, 2011) for the forest land conservation activities. The stability of land depends upon the vegetation aspects of the region. The vegetation prospers where the soil ingredients are rich. Andheri landslide region is considered to be one of the active landslide zones of east Sikkim one of the main reason is its high pH value, as recorded it has 3.05% pH (Table.1). The study observed that landslide-free zones Merung and Bhusuk has durable pH property of 4.59% and 4.71%, as compared to other landslide areas which might be helping in the growth of

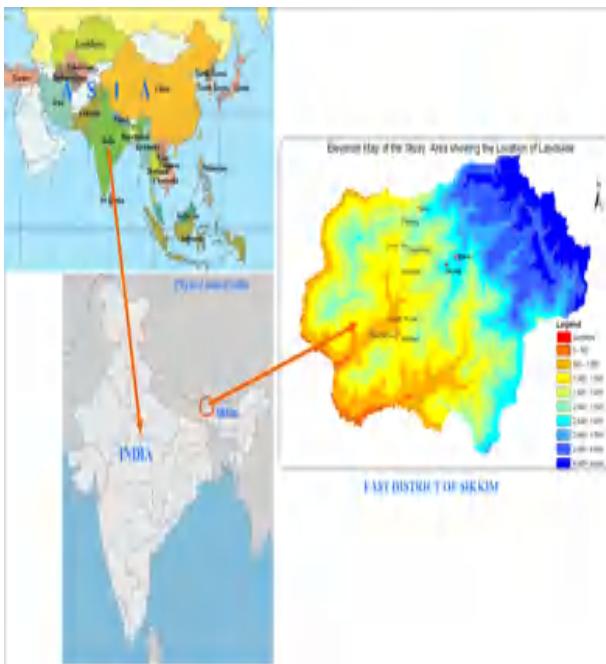
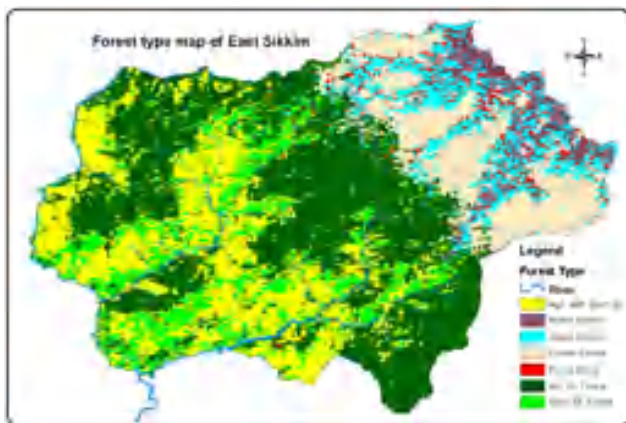
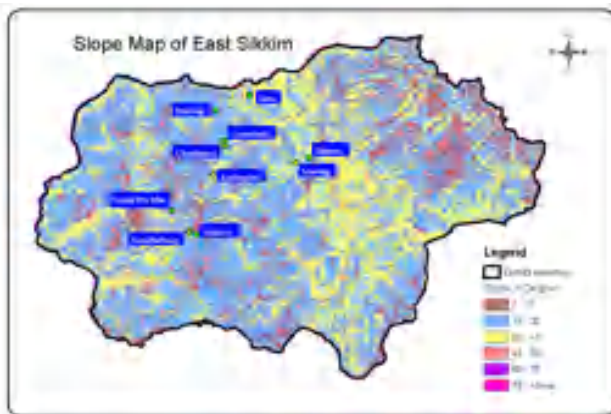


Fig. 1: Location and Elevation map of studied Eight (8) Landslides and Two (2) Landslide-free zones of East Sikkim.



A



B

Fig. 2: (A). Forest/Vegetation map of East Sikkim (B). Slope map of East Sikkim

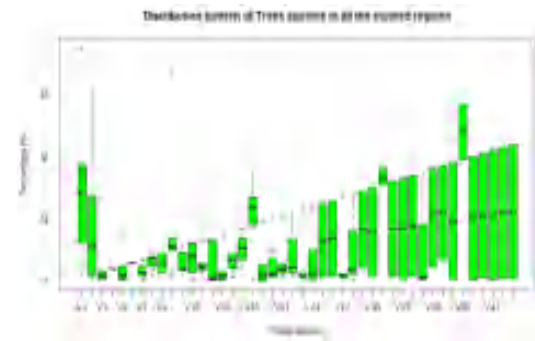


Fig.3: Distribution pattern based on Relative Density of all the Trees species in all the studied regions.

V1 *Alnus nepalensis*, V2 *Schima wallichii*, V3 *Prunus cornuta*, V4 *Daphne bholua*, V5 *Arundinaria* sp., V6 *Betula alnoides*, V7 *Erythrina stricta*, V8 *Mallotus nepalensis*, V9 *Maesa chisia*, V10 *Cryptomeria japonica*, V11 *Erythrina* sp., V12 *Terminalia myriocarpa*, V13 *Bambosa* sp., V14 *Choerospondias axillaris*, V15 *Bucklandia papulnea*, V16 *Unknown* sp., V17 *Symplocos theifolia*, V18 *Vibernum erubescens*, V19 *Cedrella kingie*, V20 *Albizzia lebbeck*, V21 *Castanopsis indica*, V22 *Eurya acuminata*, V23 *Leucosceptrum canum*, V24 *Prunus persica*, V25 *Unknown* sp., V26 *Actinodaphne obovata*, V27 *Ostodes paniculatus*, V28 *Duabanga grandiflora*, V29 *Ficus hookeri*, V30 *Saurauvia nepalensis*, V31 *Arundiaria* sp., V32 *Chukrassia tabularis*, V33 *Unknown* sp., V34 *Ficus hispida*, V35 *Bischofia javanica*, V36 *Zanthoxylum armatum*, V37 *Betula cylindrostachya*, V38 *Juglans regia*, V39 *Poa* sp., V40 *Ficus elastica*, V41 *Prunus cerasoides*, V42 *Rhus semialata*, V43 *Unknown* sp., V44 *Ficus semicordata*

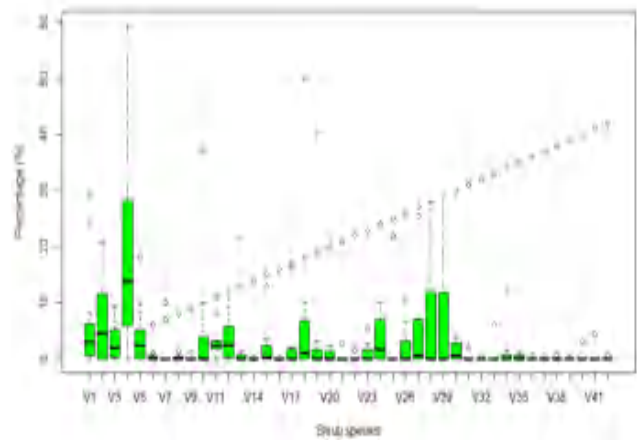
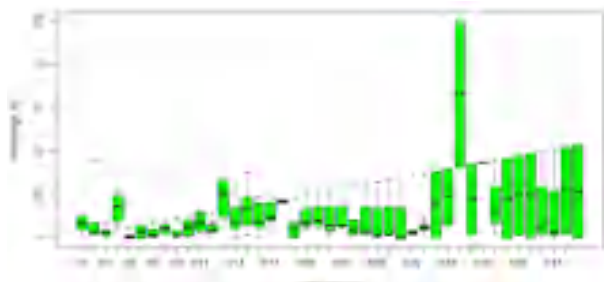


Fig.4: Distribution pattern based on Relative Density of all the Shurbs species in all the studied regions.

V1 *Thysanolaena maxima*, V2 *Fern* sp., V3 *Artemisia vulgaris*, V4 *Eupatorium cannabinum*, V5 *Osbeckia nepalensis*, V6 *Machilus edulis*, V7 *Polygonum molle*, V8 *Poa* sp., V9 *Ophiopogon intermedius*, V10 *Pilea* sp., V11 *Maesa chisia*, V12 *Pteridium aquilinum*, V13 *Ageratum conyzoides*, V14 *Rhododendron* sp., V15 *Rubus calycinus*, V16 *Bombaceae* sp., V17 *Neyraudia madagascariensis*, V18 *Urtica* sp. V19 *Brugmansia suaveolens*, V20 *Calocasia* sp., V21 *Prunus racemosa*, V22 *Imperata cylindrica*, V23 *Daphne cannabina*, V24 *Amomum subulatum*, V25 *Houttuynia cordata*, V26 *Dryopteris filixanus*, V27 *Acacia americana*, V28 *Boehmeria macrophylla*, V29 *Lantana camara*, V30 *Laportea crenulata*, V31 *Setaria palmifolia*, V32 *Tupistra nutans*, V33 *Cestrum aurantiacum*, V34 *Unknown* sp., V35 *Agave americana*, V36 *Brassaiopsis mitis*, V37 *Euphorbia pulcherrima*, V38 *Citrus* sp., V39 *Abelmoschus esculentus*, V 40 *Unknown* sp.(1), V41 *Dryoathyrium boryanum*, V42(*Large Fern* sp).



(A) (B)



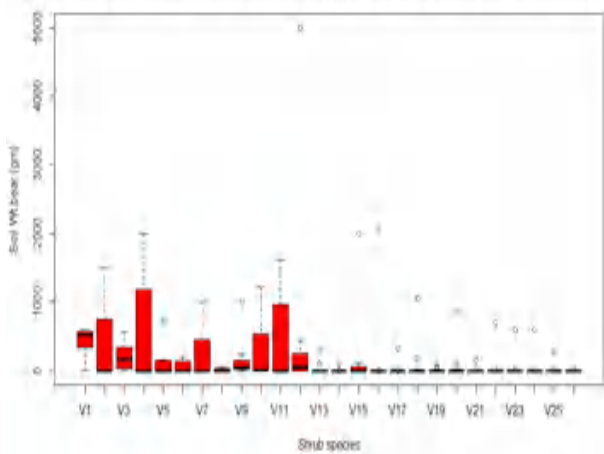
(C)

Fig. 7: Mitigative shrub *Thysanolaena maxima*: (A). Shoot (B). Expansion (C). Rhizospheric root

Fig. 5: Distribution pattern based on Relative Density of all the Herbs species in all the studied regions.

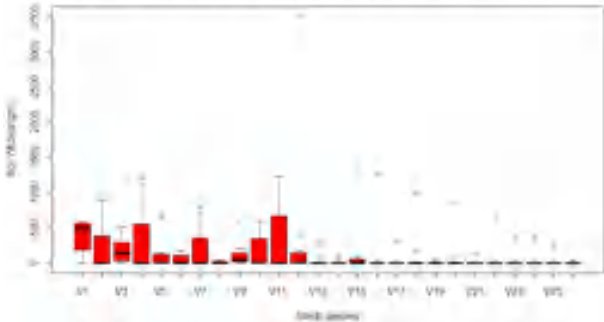
V1 *Selaginella* sp., V2 *Polystichum aculeatum*, V3 *Ophiopogon intermedius*, V4 *Poa* sp., V5 *Rubus* sp., V6 *Creep* sp., V7 *Piper retrofractum*, V8 *Pilea* sp., V9 *Floscopa scandens*, V10 *Knoxia hispida*, V11 *Glaphylopteriopsis erubescens*, V12 *Persicaria hydropiper*, V13 *Drymaria cordata*, V14 *Hydrocotyll javanica*, V15 *Pilea scripta*, V16 *Ageratum conyzoides*, V17 *Persicaria nepalensis*, V18 *Polystichum aculeatum*, V19 *Bidens pilosa*, V20 *Chenopodium ambrosioides*, V21 *Digitaria* sp., V22 *Potentilla* sp., V23 *Stellaria media*, V24 *Anaphalis contorta*, V25 *Torenia violacea*, V26 *Equisetum diffusion*, V27 *Agrostis micrantha*, V28 *Tetrastigma serrulatum*, V29 *Imperata cylindrica*, V30 *Cynodon dactylon*, V31 *Rumex nepalensis*, V32 *Oxalis corymbosa*, V33 *Mosses* sp., V34 *Digitaria sanguinalis*, V35 *Potentilla* sp.(1), V36 *Helichrysum* sp., V37 *Dioscorea dioica*, V38 *Mimosa pudica*, V39 *Urena lobata*, V40 *Fern* sp., V41 *Unknown* sp., V42 *Imperata* sp., V43 *Setaria palmifolia*.

Soil bearing strength of Shrub species during Rainy season (July-September) in all the studied regions



A

Soil bearing strength of Shrub species during Dry Season (December-January) in all the studied regions



B

Fig. 6: Boxplot graph of soil bearing strength of all the Shrub species during (A). Rainy and (B). Dry seasons.



Fig.8: Microscopic view of soil spore of *Glomus* sp.

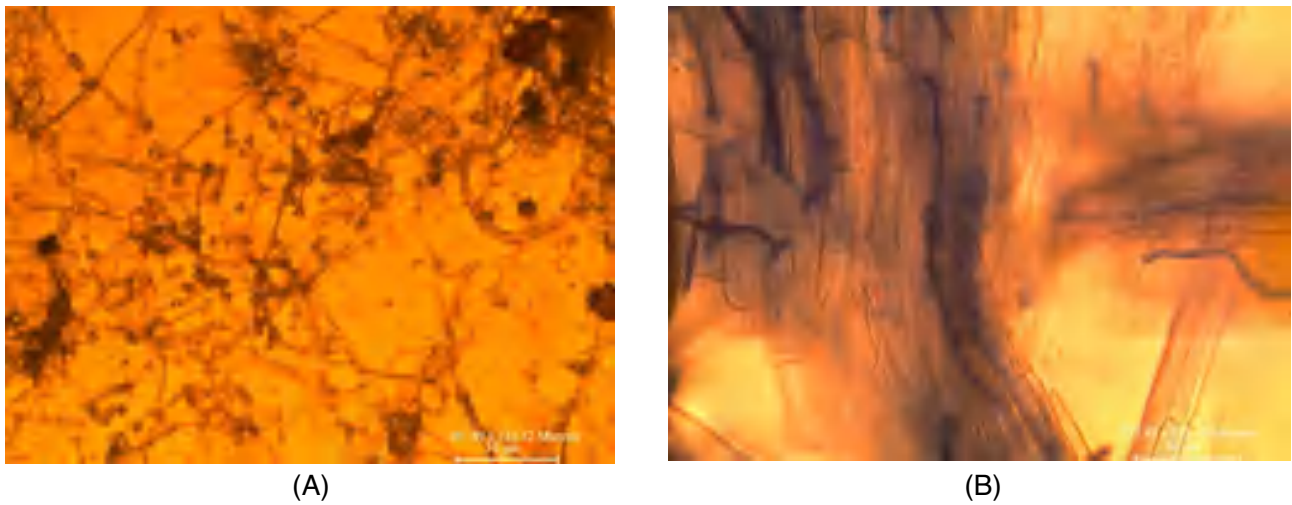


Fig. 9: Microscopic view : (A). Vesicle (B). Arbuscules

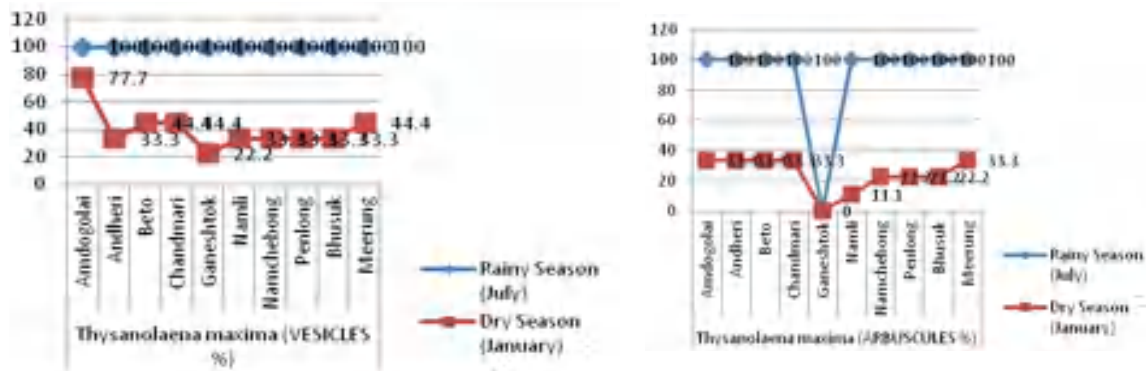


Fig.10: Graph representing colony percentage (%) ratio of rhizospheric root Vesicles and Arbuscules (VAM) of *Thysanolaena maxima* during Rainy season and Dry season in all the studied landslide zones and landslide-free zones.

Table 1: Physico-chemical nature of studied landslide areas

Name of studied region	pH	Org. C (%)	N (%)	K ₂ O (ppm)	P ₂ O ₅ (ppm)	S(ppm)	Org. Matt.(%)	E.C. m.mho/cm	Silt%	Clay%	Sand%	Texture Class
Amdogolai	5.92	1.90	0.16	67.80	8.71	7.86	3.27	0.06	6	34	42	Sandy clay
Andheri	3.05	1.28	0.11	80.90	14.15	10.80	2.21	0.05	6	42	48	Sandy clay
Beto	5.83	1.00	0.09	85.80	22.85	10.80	1.724	0.02	6	32	51	Sandy clay
Chandmari	3.27	3.23	0.28	204.14	21.76	23.57	5.57	0.09	8	41	42	Sandy clay
Ganeshtok	5.86	0.03	0.02	19.20	11.97	11.50	0.05	0.03	2	32	62	Sandy clay
Namchebong	3.21	1.35	0.12	85.32	34.82	13.74	2.33	0.04	4	40	50	Sandy clay
Namli	6.12	0.28	0.02	61.20	10.88	9.82	0.48	0.03	18	34	43	Sandy clay
Penlong	3.78	3.13	0.27	197.82	30.47	22.59	5.40	0.02	4	36	51	Sandy clay
Merung	4.59	1.38	0.12	78	15	40	2.37	0.10	40	30	30	Clay Loam
Bhusuk	4.71	1.41	0.12	80	12	40	2.43	0.09	40	20	40	Clay Loam

Table 2: Vegetation distribution patterns of studied Landslide and Landslide-free zones.

TREES VEGETATION : Distribution patterns of seven major species in Frequency (Frq.), Relative Frequency (Rel.Frq.) and Importance Value (Imp.Val.)																					
Studied zones	<i>Alnus nepalensis</i>			<i>Schima wallichii</i>			<i>Choerospondias axillaris</i> (in %)			<i>Bucklandia papulnea</i>			<i>Leucosceptrum canum</i>			<i>Bambosa sp.</i>			<i>Castanopsis indica</i> (in %)		
	Frq.	Rel. Frq.	Imp. Val.	Frq.	Rel. Frq.	Imp. Val.	Frq.	Rel. Frq.	Imp. Val.	Frq.	Rel. Frq.	Imp. Val.	Frq.	Rel. Frq.	Imp. Val.	Frq.	Rel. Frq.	Imp. Val.	Frq.	Rel. Frq.	Imp. Val.
Amdogolai	50	7.49	6.90	100	14.9	12.1	-	-	-	-	-	-	-	-	50	7.49	1.89	50	7.49	4.21	
Andheri	50	7.49	12.7	83.33	12.4	57.1	33.3	4.99	2.3	-	-	-	-	-	66.66	9.99	4.6	50	7.49	5.9	
Beto	100	14.9	41.33	-	-	-	16.6	2.49	1	-	-	-	-	-	33.33	4.99	3.34	-	-	-	
Chandmari	100	14.9	52.3	16.66	2.49	1.4	16.6	2.49	1.10	33.33	4.99	1.9	16.66	2.49	1.5	16.66	2.49	1	-	-	-
Ganeshtok	50	7.49	14.86	-	-	-	-	-	-	16.66	2.49	1.68	-	-	-	-	-	-	-	-	-
Namli	83.35	12.5	28.08	50	7.49	11.3	33.3	4.99	7.87	-	-	-	-	-	-	-	-	-	16.66	2.50	3.38
Namchebong	83.33	12.4	36.6	100	14.9	30.2	-	-	-	-	-	-	-	-	50	7.49	3.6	66.66	9.99	6.2	
Perlong	66.66	9.99	11	-	-	-	-	-	-	16.66	2.49	1.8	16.66	2.49	1.5	-	-	-	16.66	2.49	2.4
Bhusuk	66.66	9.99	12.6	-	-	-	-	-	-	16.66	2.49	1.39	16.66	2.49	1.68	-	-	-	16.66	2.49	2.81
Merung	66.66	9.99	12.9	-	-	-	-	-	-	16.66	2.49	1.90	16.66	2.49	1.36	-	-	-	16.66	2.49	1.58
SHRUBS VEGETATION: Distribution pattern of seven major species in Frequency (Frq.), Relative Frequency (Rel.Frq.) and Importance Value (Imp.Val.)																					
Studied zones	<i>Thysanolaena maxima</i> (in %)			<i>Eupatorium cannabinum</i> (in %)			<i>Amomum subulatum</i>			<i>Pteridium aquilinum</i> (in %)			<i>Osbeckia nepalensis</i> (in %)			<i>Lantana camara</i> (in %)			<i>Acacia pinnata</i> (in %)		
	Frq.	Rel. Frq.	Imp. Val.	Frq.	Rel. Frq.	Imp. Val.	Frq.	Rel. Frq.	Imp. Val.	Frq.	Rel. Frq.	Imp. Val.	Frq.	Rel. Frq.	Imp. Val.	Frq.	Rel. Frq.	Imp. Val.	Frq.	Rel. Frq.	Imp. Val.
Amdogolai	33.33	4.99	4.99	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Andheri	33.33	4.99	2.2	66.66	9.99	12.9	33.3	4.99	4.18	33.33	4.99	3.2	-	-	-	50	7.49	6.2	16.66	2.49	3
Beto	50	7.49	3	50	7.49	6.5	-	-	-	33.33	4.99	5.28	83.33	12.4	7.4	-	-	-	-	-	-
Chandmari	16.66	2.49	1.84	66.66	9.99	19.8	13.3	4.99	2.24	16.66	2.49	1	50	7.49	4.0	-	-	-	-	-	-
Ganeshtok	-	-	-	100	14.9	24.8	-	-	-	-	-	-	83.33	12.4	5.92	-	-	-	-	-	-
Namli	33.33	4.99	2.75	33.33	4.99	3.11	-	-	-	-	-	-	-	-	-	-	-	-	66.66	9.99	11.8
Namchebong	50	7.49	5.24	33.33	4.99	4.21	16.6	2.49	2.98	16.66	2.49	3.18	83.33	12.4	10.2	-	-	-	-	-	-
Perlong	66.66	9.99	5.56	16.66	2.49	10	66.6	9.99	6.73	16.66	2.49	1.29	16.66	2.49	1.66	-	-	-	16.66	2.49	1
Bhusuk	33.33	4.99	9.8	66.66	9.99	9.61	33.3	4.99	2.23	33.33	4.99	2.46	-	-	-	50	7.49	6.60	16.66	2.49	3.23
Merung	33.33	4.99	11.54	66.66	9.99	7.94	33.3	4.99	2.65	33.33	4.99	2.57	-	-	-	50	7.49	8.42	16.66	2.49	2.31
HERBS VEGETATION: Distribution pattern of seven major species in Frequency (Frq.), Relative Frequency (Rel.Frq.) and Importance Value (Imp.Val.)																					
Studied zones	<i>Cynodon dactylon</i> (in %)			<i>Drymaria cordata</i> (in %)			<i>Ageratum conyzoides</i> (in %)			<i>Panicum hydropiper</i>			<i>Panicum nepalensis</i> (in %)			<i>Ophiopogon intermedius</i> (in %)			<i>Hydrocotyl javanica</i> (in %)		
	Frq.	Rel. Frq.	Imp. Val.	Frq.	Rel. Frq.	Imp. Val.	Frq.	Rel. Frq.	Imp. Val.	Frq.	Rel. Frq.	Imp. Val.	Frq.	Rel. Frq.	Imp. Val.	Frq.	Rel. Frq.	Imp. Val.	Frq.	Rel. Frq.	Imp. Val.
Amdogolai	83.33	12.49	5.06	66.66	9.99	6.59	50	7.49	4.24	33.33	4.99	2.67	100	14.99	12.9	16.66	2.49	1.2	33.33	4.99	4
Andheri	100	14.99	7.87	83.33	12.4	11.17	50	7.49	5.10	16.66	2.49	3.24	-	-	-	-	-	-	-	-	-
Beto	100	14.99	6.34	-	-	-	-	-	-	-	-	-	33.33	4.99	2.80	50	7.49	3.55	66.66	9.99	10.4
Chandmari	100	14.9	6.38	66.66	9.99	14	-	-	-	33.33	4.99	2.61	83.33	12.4	7.27	33.33	4.99	1.76	-	-	-
Ganeshtok	16.66	2.49	2.59	83.33	12.4	13.3	16	2.49	1.10	-	-	-	50	7.49	4.75	50	7.49	3.57	50	7.49	5.37
Namli	100	14.99	6.57	83.33	12.4	6	83	12.4	10.5	66.66	9.99	4.43	-	-	-	33.33	4.99	2	83.33	12.4	6.69
Namchebong	100	14.99	6.66	16.66	2.49	3.55	-	-	-	16.66	2.49	2.49	-	-	-	83.33	12.4	5.45	16.66	2.49	2.35
Perlong	16.66	2.49	1.46	33.33	4.99	10.5	-	-	-	33.33	4.99	2.37	50	7.49	7.78	83.33	12.4	5.17	16.66	2.49	1
Bhusuk	100	14.9	8.21	100	14.9	12.8	-	-	-	100	14.9	6.88	100	14.9	8	100	14.9	6	83.33	12.4	5.44
Merung	100	14.99	4.38	-	-	-	-	-	-	-	-	-	33.33	4.99	4.68	50	7.49	4.61	66.66	9.99	11.96

Table 3: Rhizospheric soil spores colony distribution (%) of *Thysanolaena maxima* in studied landslide zones and landslide-free zones : Amdogolai (1), Andheri (2), Beto (3), Chandmari (4), Ganeshtok (5), Namchebong (6), Namli (7), Penlong (8), Merung (9), Bhusuk (10)

Spore Species	1		2		3		4		5		6		7		8		9		10	
	July	Jan	July	Jan	July	Jan	July	Jan	July	Jan	July	Jan	July	Jan	July	Jan	July	Jan	July	Jan
<i>Glomus sp.</i>	100	25	37.5	12.5	12.5	0.0	100	75	12.5	0.0	0.0	0.0	100	0.0	25	12.5	37.5	12.5	62.5	12.5
<i>Scutellospora ceradensis</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Acaulospora sp.</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

healthy vegetation in the region, manifesting more towards the stability of land mass. Moreover, it was interesting to note that landslide-free zones Merung and Bhusuk are showing no significant difference in ingredient values except Sulphur (S), which is recorded with a value of 40%, while others eight landslide areas have very low Sulphur value in their ingredient. This indicated that Sulphur richness is very useful factor for the soil fertility and stability of the regions. As reported previous from researches on the crop plants, the plants have become increasingly dependent on the soil to supply the sulphur which is needed by plant for the synthesis of proteins and a number of essential vitamins and cofactors.

Even though the elevation level is recorded as high in landslide-free zones Merung and Bhusuk with 2500-3000 meters, the occurrence of landslide activities are not recorded in these regions. One of the main factors responsible for non-occurrence of landslide in these regions is the presence of healthy vegetation (Table 2) dominated maximally by mitigative species *Thysanolaena maxima*. In addition, to its role as a mitigative species, *Thysanolaena maxima* also has an important commercial value; the village farmers collect shoots of plant and sell it as brooms in market. However, this study also helps to recognize the new optimistic role and value of *Thysanolaena maxima* towards bioengineering the stability of land surface especially during rainy seasons. It is well understood that higher the elevation degree higher will be the slope aspects. Elevation and slope are considered to be major factors of landslides (Mahalingam *et al.* 2016). In case of the studied landslide-free zones the slope aspects were found in between 30° - 45° (Fig.2B), which is higher than that of all other landslide prone zones. It indicates that high elevation and slope aspects alone are not the sole causal factors but also composition and formation of vegetation types

play a role in stabilization of land mass. Grass species like *Cynodon dactylon*, belonging to Poaceae family was found as most abundant species among the herbs which and being structurally attached to the soil mass also have an important role to play in lessening the effect of rain drops during rainfall.

Analyses of plants with excellent soil binding characters are difficult because of the huge diversity of the plant kingdom. It requires considerable concerted effort and specific methods to understand the plant root shear strength. However, in this study the soil binding capacity of roots were analysed from every inhabiting shrub plants species in the landslide areas including landslide-free zones. *Thysanolaena maxima* were found to be most efficient shrub species to manifest a role in binding, holding and stabilization of the land mass (Table 3). There were other species such as *Eupatorium cannabinum* (Asteraceae) and *Artemesia vulgaris* (Asteraceae) which exhibited good soil bearing root characters but due to its unsuitable morphological architectures and behaviours such as, both of these shrub species prefers to grow in marshes, moist and wet environment and therefore, if used, will bring about formation of wet or moist soil mass which will be prone to be eroded easily. While *Thysanolaena maxima* maximumly prefer any soil conditions for its survival. (Fig. 3,4 and 5). *Eupatorium cannabinum* and *Artemesia vulgaris* have tap root system, which intrinsically does not promote strong fibrous adventitious roots as in *Thysanolaena maxima* (Poaceae), as a consequence, it will have lower root reinforcement system and will not be able to adhere and grip the soil particles and stabilize the land mass. Also, they do not grow in root communion or cluster formation as it is in *Thysanolaena maxima*. The species therefore, were not suitable as mitigative species. One of the paramount behaviour of

Table 4: Bioengineering of shrub species

Sl. No./ Fields	Name of the Shrub Species	Total Height (in cm)	Root N length G (in cm)	Brdh. (root in cm)	Shoot height (in cm)	Breadth (Canopy coverage in cm)	Soil bearing capacity (During Rainy season, July- September)	Soil bearing Capacity (During dry season Dec January)
1	<i>Osbeckia nepalensis</i> Hooker	152.4	36.57	40	124.96	88.39	245 gm	200 gm
2	<i>Eupatorium cannabinum</i> L.	100.58	30.48	48.76	79.24	39.62	270 gm	200 gm
3	<i>Daphne cannabina</i> Wall.	118.87	42.67	39.62	121.92	100.58	265 gm	190 gm
4	<i>Artemisia vulgaris</i> Willdenow	94.48	30	42.67	88.39	42.67	251 gm	180
1	<i>Daphne cannabina</i> Wall.	140.20	46	24	112.77	40	50gm	46 gm
2	<i>Houttuynia cordata</i>	94.48	22	28	100.58	56	198gm	168 gm
3	<i>Amomumsubulatum</i> Roxb.	158.49	45	52	121.92	100.58	725 gm	650 gm
4	<i>Osbeckia nepalensis</i> Hooker	109.72	19	26	64	33	35 gm	26 gm
5	<i>Maesa chisia</i> Buch- Ham. ex D. Don	103.63	50	28	72	70.10	300 gm	280 gm
6	<i>Artemisia vulgaris</i>	125.27	23	29	121.92	91.44	450 gm	400 gm
7	<i>Eupatorium cannabinum</i> L.	100.58	24	15	82.29	45	575 gm	515 gm
8	<i>Pteridium aquilinum</i>	164.59	16	17	125.27	64	100 gm	85 gm
9	<i>Dryopterisoreades</i> Fomin	149.35	12	25	52	45	125 gm	85 gm
10	<i>Thysanolaena maxima</i> (Roxb.) Kuntze	219.45	30	21	200	106.68	572 gm	565 gm
1	<i>Eupatorium cannabinum</i> L.	91.44	24	24	70.10	53	129 gm	112 gm
2	<i>Osbeckia nepalensis</i> Hooker	219.45	64.	16	125.27	79	1kg(1000gm)	600 gm
3	<i>Dryopters oreades</i> Fomin	253	53	46	2m	73.15	2kg(2000gm)	1300 gm
4	<i>Artemisia vulgaris</i>	281	57	71	226	182.88	5kg (5000gm)	3500gm
5	<i>Thysanolaena maxima</i> (Roxb.) Kuntze	216.40	35	22	2m	112.77	582 gm	576 gm
6	<i>Rubus</i> sp.	232	52	42	182.88	53	380gm	340 gm
7	<i>Polygonum molle</i>	188.97	46	60.96	124.96	64	2050gm	1270 gm
8	<i>Urticasp.</i> L.	407.26	83	21	385.92	112.77	325gm	290 gm
1	<i>Boehmeria macrophylla</i> Hornem	236	109.72	50	131.06	87	1060gm	1000 gm
2	<i>Eupatorium odoratum</i> L.	212	22	9	155.44	43	50gm	40 gm
3	<i>Eupatorium cannabinum</i> L.	94.48	20	18	100.58	26	30gm	23 gm
4	<i>Thysanolaena maxima</i> (Roxb.) Kuntze	217.32	35	23	2m	106.68	510 gm	500 gm
5	<i>Artemisia vulgaris</i>	182.88	30.48	14	158.49	29	105gm	95 gm
6	<i>Osbeckia nepalensis</i> Hooker	161.54	28	20	137.16	65	50gm	42 gm
1	<i>Boehmeria macrophylla</i> Hornem	164.59	55	19	106.68	55	180gm	172 gm
2	<i>Thysanolaena maxima</i> (Roxb.) Kuntze	216.40	30	21	200	97.53	520gm	200 gm
3	<i>Neyrardia madagascariensis</i>	200	58	24	149.35	99	865gm	847 gm
4	<i>Amomums ubulatum</i> Roxb.	149.35	60.96	27	85.34	67.05	120gm	105 gm
5	<i>Maesa chisia</i>	230.48	18	5	176.78	134.11	100gm	78 gm
6	<i>Osbeckia nepalensis</i> Hooker	319	67.05	30.48	250	59	45gm	32 gm
7	<i>Eupatorium cannabinum</i> L.	134.11	15	8	118.87	20	55gm	48gm
1	<i>Datura suaveolens</i>	146.30	20	25	94.79	27	75gm	57 gm
2	<i>Curcuma longa</i> Linn	118.87	14	5	103.63	42.67	170gm	146 gm
3	<i>Girardinia palmate</i> Forsk	73.15	34	9	37	42	126gm	106 gm
4	<i>Thysanolaena maxima</i> (Roxb.) Kuntze	217.93	33	21	2m	115.82	575 gm	562 gm
5	<i>Cyathea australis</i>	88.39	33.52	12	173.73	60.96	695gm	664 gm
6	<i>Eupatorium cannabinum</i> L.	94.79	36.57	7	64	64	30gm	18 gm
7	<i>Artemisia vulgaris</i>	121.92	20	35	103.63	17	75gm	56 gm
1	<i>Girardinia palmate</i> Rorsk Gaud.	152.92	23	118	97	65	1kg	600 gm
2	<i>Houttuynia cordata</i>	207	70	154	100	55	1kg 500gm	1000 gm
3	<i>Eupatorium cannabium</i>	103.63	28	59	62	62	350gm	315 gm
4	<i>Dryopterisaemula</i> (Ait.) Kuntze	155.44	36	28	143.25	42	2kg	1200 gm
5	<i>Ammomum subulatum</i>	143.25	22	40	128.01	53	151gm	125 gm
6	<i>Datura suaveolens</i>	220	100	200	204	73.15	195gm	157 gm
7	<i>Thysanolaena maxima</i> (Roxb.) Kuntze	217.01	32	21	2m	115.82	565 gm	555 gm
8	<i>Rubus moluccanus</i> Linn.	137.16	100	300	109.72	100	55gm	41 gm
9	<i>Osbeckianepalensis</i> Hooker	158.49	36.57	40	128.01	71	149gm	130 gm
10	<i>Daphne cannabina</i> Wall.	137.16	27	3	112.77	26	1223mg	500 mg
11	<i>Rubus</i> sp.Smith	140.20	60.96	40	79.24	25	1545mg	1000mg
1	<i>Ficus nemoralis</i> Wall.	106	82.29	67.05	134.11	76.20	600gm	350 gm
2	<i>Lantana camara</i> Linn.	246.88	137.16	33.52	155.44	121.92	600gm	360 gm
3	<i>Ageratum conyzoides</i> L.	100.58	16	27	64	103.63	275gm	243 gm
4	<i>Thysanolaena maxima</i> (Roxb.) Kuntze	106.68	15	18	64.31	52	180gm	179 gm
5	<i>Neyrardia madagascariensis</i>	200	18	27	182.88	56	97gm	87 gm
6	<i>Eupatorium odoratum</i> L.	212	28	9	192.02	57	60gm	40 gm
7	<i>Dryopterisoreades</i> Fomin	76.20	20	30	58	57	100gm	87 gm
8	<i>Fern</i> sp.	146.30	15	1	124.96	109.72	20gm	12 gm
9	<i>Eupatoriumcannabinum</i> L.	106.68	18	18	64.31	38	170gm	140 gm
10	<i>Dryopterisaemula</i> (Ait.) Kuntze	103.63	29	39.62	73.15	39.62	360gm	310 gm
1	<i>Girardinia palmate</i> Rorsk Gaud.	161.54	26	116	100	67	900 gm	800 gm
2	<i>Houttuynia cordata</i>	200	60	147	100	52	1kg 300gm	900gm

M E R U N G	1	<i>Girardinia palmate</i> Rorsk Gaud.	161.54	26	116	100	67	900 gm	800 gm
	2	<i>Houttuynia cordata</i>	200	60	147	100	52	1kg 300gm	900gm
	3	<i>Eupatorium cannabinum</i>	121.92	27	57	60	61	345gm	298 gm
	4	<i>Dryopteris aemula</i> (Ait.) Kuntze	152.4	34	29	158.49	43	2kg	1000 gm
	5	<i>Ammomum subulatum</i>	140.20	20	45	124.96	54	155gm	143 gm
	6	<i>Datura suaveolens</i>	200	45.72	67.05	200	70.10	200gm	180 gm
	7	<i>Thysanolaena maxima</i> (Roxb.) Kuntze	216.40	37	24	200	80	600 gm	570 gm
	8	<i>Rubus moluccanus</i> Linn.	128.01	36.57	91.44	106.68	51.81	58gm	41 gm
	9	<i>Osbeckia nepalensis</i> Hooker	152.4	48.76	39	137.16	70	152gm	141 gm
	10	<i>Daphne cannabina</i> Wall.	131.06	29	6	115.82	28	1000 gm	600 mg
	11	<i>Rubus sp.</i> Smith	146.30	91.44	50	82.29	29	1600gm	1200mg
B H U S U K	1	<i>Girardinia palmate</i> Rorsk Gaud.	158.49	22	110	88	69	800 gm	700 gm
	2	<i>Houttuynia cordata</i>	150	55	137	100	52	1kg 300gm	600gm
	3	<i>Eupatorium cannabinum</i>	121.92	27	57	60	60	340gm	290 gm
	4	<i>Dryopteris aemula</i> (Ait.) Kuntze	167.64	37	29	121.92	44	2kg	800 gm
	5	<i>Ammomum subulatum</i>	146.30	25	43	137.16	58	160gm	120 gm
	6	<i>Datura suaveolens</i>	250	54.86	76.2	200	76.2	200gm	165 gm
	7	<i>Thysanolaena maxima</i> (Roxb.) Kuntze	213.36	40	26	250	90	500 gm	460 gm
	8	<i>Rubus moluccanus</i> Linn.	137.16	39.62	91.44	115.82	54.86	60gm	40 gm
	9	<i>Osbeckia nepalensis</i> Hooker	158.49	54.86	40	128.01	75	155gm	135 gm
	10	<i>Daphne cannabina</i> Wall.	128.01	30	7	97.53	300	800gm	590 mg
	11	<i>Rubus sp.</i> Smith	137.16	103.63	53	88.39	33	1620gm	1230mg

Table 5: Rhizosphere fungal associations of *Thysanolaena maxima*

Sample ID	Accession No.	Nature of zone	Rhizosphere fungus
<i>T.maxima</i> (Amdogolai)	-	Landslide	-
<i>T.maxima</i> (Bhusuk) pen tm1	KY766265	Landslide-free	<i>Ascomycota</i> sp.
TmBeto140913	KY766266	Landslide	<i>Ascomycota</i> sp.
TmAndheri170913	KY766267	Landslide	<i>Ascomycota</i> sp.
TmNamcebong180913	-	Landslide	-
TmChandmari180913	-	Landslide	-
TMnamli22102013	KY766264	Landslide	<i>Cladophialophora</i> sp.
TmaximaMerung201213	KY766268	Landslide-free	<i>Ascomycota</i> sp.

Thysanolaena maxima is that with its maturity it grows in cluster formation and covers an expanding area of land mass thus enhancing the coverage area. The canopy formation is also one of the major factors to authenticate *Thysanolaena maxima* as a mitigative species. As recorded (Table 4.) growing canopy coverage of *Thysanolaena maxima* extended up to 115.82 cm and the depth of gripping roots goes down up to 40 cm and laterally upto 26 cm. Therefore the presence of *Thysanolaena maxima* will make a positive impact towards the stability of land mass if active bioengineering plantation projects are undertaken.

The occurrence frequency rate of *Alnus nepalensis* between Penlong landslide (Non-active) and two landslide-free zones Merung and Bhusuk when compared is the same with 66.66% for all above three zones, yet after some years,

landslide eruptions occurred in case of Penlong landslide zone while Merung and Bhusuk zones were unaffected. The main reason is the distribution pattern of vegetation in Penlong. Among all plant species the presence of *Thysanolaena maxima* was rare which led to re-occurrence of landslide activities after some years. However, in case of Merung and Bhusuk the vegetation was maximally dominated by *Thysanolaena maxima*.

It has been observed that compared to landslide-free zones all the studied landslide regions are recorded under low elevation zones. But, the eruptions and prevalence of landslide activities are always found more in these lateral zones, the main reason behind its eruptions is the distribution pattern of plant vegetation. In these low elevation regions the presence of *Thysanolaena maxima* is

very negligible as compared to landslide-free zones. All the landslide studies on Himalayan regions have recorded *Alnus nepalensis* as a dominant tree plants, this study also found the species as dominant among all the tree plants. Since, *Alnus species* do not have potential root shear strength to stabilize the land mass, the presence of other dominant trees like *Schima wallichias* noted in this study, is a positive indication as it can function as a major alternative mitigative tree species because of its more rigid structure in contrast to *Alnus* sp. which can be easily broken by mechanical forces of much smaller magnitude.

Along with the identification of this efficient shrub species *Thysanolaena maxima*, it has also become important to understand the soil microbial VAM and fungal species associations in the rhizosphere due to the important role it plays in the nutritional availability of the mitigative plant for its survival. The VAM study found that most of the regions are dominated by *Glomus* sp. It has an ability to produce more spores than *Gigaspora* and *Scutellospora species* (Fig. 8, 9 and 10). (Suresh and Nagarajan, 2010). Spores of both genera *Scutellospora* and *Acaulospora sp.* were not found confined in the rhizosphere of *Thysanolaena maxima*. These fungi are the foremost occupiers of soil and help in soil sustenance during the various adverse conditions, breaking down organic matter, natural decomposing of soil leading towards the regulation of carbon cycles and maintenance of the balance, conversion into biomass, carbon and organic acids, many of which are essential and important nutrient parameters for plants. This study (Table 5) found that fungi such as *uncultured Ascomycota species*, were found to be prevalent and dominant within the roots of *Thysanolaena maxima*. At times, *Cladophialophora* sp. were also found inhabiting within the rhizospheric root. *Cladophialophora sp.* belongs to genus of black yeast-like fungi and known for its nature of biodegradation especially for BTEX compounds such as toluene and ethyl benzene (Prenafeta Boldu *et al.* 2002). The presence of this fungus will perhaps help to perform biodegradation process of severe pollutant agents enhancing the ecology and environment. Therefore perhaps an additional benefit is that bioengineering plantations of *Thysanolaena maxima* will also have an ability to combat polluting xenobiotic compounds in the environment.

This study therefore indicates that bioengineering of affected Himalayan regions of Sikkim with *Thysanolaena maxima* will not only help to mitigate landslides which will have far reaching long term impact on ecology and environment as well as, also help to control the pollutants and therefore help to sustain the pristine nature of these fragile Himalayan ecosystems.

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