Seed germination patterns of some Asteraceae species from two regions with contrasting precipitation in north-central Nepal

A Dissertation Submitted for the Partial Fulfillment of the Requirement for the Master's Degree in Science, Central Department of Botany, Institute of Science and Technology, Tribhuvan University

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DECLARATION

I, Sarada Dhakal, hereby declare that this dissertation entitled "**Seed germination patterns of some Asteraceae species from two regions with contrasting precipitation in north-central Nepal**" which is being submitted to the Central Department of Botany, Institute of Science and Technology, Tribhuvan University, Nepal for the award of degree of Master of Science in Botany is research carried out by me under the supervision of **Associate Professor Dr. Anjana Devkota** and co-supervisor **Professor Dr. Bharat Babu Shrestha**. The information acquired from the published and unpublished work of others has been acknowledged in the text. Further, a list of references has been given at the end of the thesis. In the sense, this research is original and genuine, and has not been submitted earlier partially, fully, or in any other form to any university or institute here or elsewhere, for the reward of any degree.

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LETTER OF RECOMMENDATION

This is to certify Ms. Sarada Dhakal has completed this thesis dissertation work entitled "**Seed germination patterns of some Asteraceae species from two regions with contrasting precipitation in north-central Nepal**" under our supervision. The results of this dissertation have not been been presented or submitted anywhere else for the academic degree. It is hereby recommended for the acceptance of this dissertation as a partial fulfillment of the Master's Degree in Botany at the Institute of Science and Technology, Tribhuvan University, Kathmandu, Nepal.

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LETTER OF APPROVAL

The M.Sc. dissertation entitled as "**Seed germination patterns of some Asteraceae species from two regions with contrasting precipitation in north-central Nepal**" submitted at the Central Department of Botany, Tribhuvan University by Ms. Sarada Dhakal has been accepted for the partial fulfillment of her Master's Degree in Botany (Ecology and Resource Management).

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ABBREVIATION AND ACRONYMS

ACA	Annapurna Conservation Area
ANOVA	Analysis of Variance
°C	Degree Centigrade
DNPWC	Department of National Parks and Wildlife Conservation
GP	Germination Percentage
m asl	meter above sea level
MGT	Mean Germnation Time
MPa	Mega Pascal
ND	Non-dormant Dormancy
PD	Physiological Dormancy
PEG 6000	Poly Ethylene Glycol 6000
SD	Standard Deviation
SE	Standard Error
SPSS	Statistical Package for Social Sciences
TI	Timson's Index

ABSTRACT

Germination is an important seed feature connected to a species' regeneration niche, and it can influence the distribution and abundance of species in communities. The germination of seeds in plants is governed by several environmental processes, leading to less research about their growth, adaptation, and changes in their germination behavior influenced by climate change. Present study compared germination ecology of three species of Asteraceae from moist region (*Cremanthodium reniforme*, *Dubyaea hispida*, *Synotis alata*) and closely related three species from dry region (*Cremanthodium arnicoides, Synotis penninervis* and *Taraxacum eriopodum* of the Annapurna Conservation Area in north-central Nepal. Seeds were germinated under different light (12-h photoperiod and continuous dark), temperature (low: 25/15℃ and high: 30/20℃) and water stress (0, -0.25, -0.5, -0.75 and -1 MPa) to determine germination percentage, Timson’s index (germination rate) and mean germination time (MGT). Higher seed mass was found in *Cremanthodium arnicoides* and larger seed size was found in *Dubyaea hispida*. Seeds of all species germinated in both photoperiod and continuous dark condition. At low temperature and all levels of water stresses, *Cremanthodium arnicoides* and *Synotis penninervis* exhibited higher Germination Percentage (%), while *Cremanthodium reniforme* showed higher Mean Germination Time (MGT) and *Taraxacum eriopodum* had higher values for Timson’s Index (TI). However, at high temperature significantly higher germination percentage was found in *Dubyaea hispida* and *Synotis alata*, higher MGT was found in *Synotis penninervis* and higher TI was found in *Dubyaea hispida*. No germination was recorded in five species below -0.5 MPa water potential but seeds of *Cremanthodium arnicoides* germinated up to -0.75 MPa. These finding give a clear indication about the germination traits of the species from wet and dry regions, while also providing a clear understanding about their germination patterns, survival of the plants, and effect of climate change.

Key words: *Annapurna Conservation Area*, *Environmental tolerance*, *Himalaya precipitation gradient*, *Mean Germination Time*, *Timson’s Index*

शारसं

बीउ अंकुरण स्थापनाको सबैभन्दा आधारभूत अवधि हो। यो एक अपरिवर्तनीय तथा जटिल प्रकिया हो। यस अध्ययको मुख्य उदेश्य भनेको नेपालको उत्तर मध्य भागमा रहेको अन्नपूर्ण संरक्षण क्षेत्रको ओसिलो भागमा पाइने Asteraceae को तीन प्रजातिहरु *Cremanthodium reniforme*, *Dubyaea hispida*, *Synotis alata* र यि प्रजातिसंग नजिकको सम्बन्ध रहेको सुख्खा क्षेत्रमा पाइने तीन प्रजाति *Cremanthodium arnicoides*, *Synotis penninervis* र *Taraxacum eriopodum* को अंकुरण परिस्थितिको भिन्नताबारे अध्ययन गर्नु हो।अंकुरण प्रतिशत, टिम्सन्स इन्डेक्स र औसत अंकुरण समय निर्धारण गर्न विभिन्न प्रकाश (१२ घन्टा Photoperiod र लगातार अध्यारो), तापक्रम कम (२५/१५°C) र उच्च (३०/२०°C) र water stress मा बीउ अंकुरित गरिएको थियो। उच्च बीउको मास *Cremanthodium arnicoides* र ठूलो बीउको आकार *Dubyaea hispida* मा पाइयो। सबै प्रजातिका बीउहरू Photoperiod र निरन्तर अध्यारो अवस्थामा अंकुरित भए। कम तापक्रम र सबै तहको water stress मा *Cremanthodium arnicoides* र *Synotis penninervis* ले उच्च अंकुरण प्रतिशत प्रदर्शन गर्यो । यध्यपी उच्च तापक्रममा *Dubyaea hispida* र *Synotis alata* मा उच्च अंकुरण प्रतिशत पाइयो। -0.5 MPa पानीको सम्भाव्यता भन्दा कम पाँच प्रजातिहरूमा कुनै अंकुरण रेकर्ड गरिएको थिएन तर *Cremanthodium arnicoides* को बीउहरू -0.75MPa सम्म अंकुरित भए। यी निष्कर्षहरूले ओसिलो र सुख्खा क्षेत्रहरूबाट प्रजातिहरूको अंकुरण विशेषताहरुको बारेमा स्पष्ट संकेत दिन्छ, साथै तिनीहरूको अंकुरण ढाचा र जलवायु परिवर्तनको प्रभावको बारेमा स्पष्ट बुझाइ प्रदान गर्दछ।

CHAPTER 1: INTRODUCTION

1.1 Background

Ecology of seeds is the study of how plants use various ecological techniques to ensure their ability to reproduce through seed. Seed ecology involve diverse strategies just like seed output period, mechanism of seed dispersal, environmental situations that access dormancy and activate germination, seed size and the component that impact successful seedling formation (Copeland and McDonald, 2001).

In the life cycle of the majority of plants, seed germination marks the change from the seed to seedling stages (Harper, 1977). Germination is an irreversible process that must be timed to take place when the conditions are right for following seedling establishment (Fenner and Thompson, 2005; Poschlod *et al*., 2013). Two important abiotic factors such as temperature and water regulate seed germination (Walck *et al*., 2011; Arana *et al*., 2015). Temperature and water availability restrained the timing of seed germination (Briceno *et al*., 2015). Further, germination can be influenced by phylogeny (Leishman *et al*., 1995), seed size (Norden *et al*., 2009), seed dispersal (Levey *et al*., 2005), life form (Rees, 1994) and environmental signals (Jurado and Flores, 2005). However, the most important environmental factor is water, which regulates the seedling emergence (Bradford, 2017).

Seed dormancy condition is changed by temperature, which is a major environmental element that can control the breaking of dormancy as well as the initiation of secondary dormancy (Baskin and Baskin, 1998). In germination ecology, dormancy breaking and seed germination are different events which happen in response to environmental surroundings.

It is challenging to define a common "alpine" germination strategy in the alpine environment due to the high species and microhabitat diversity, which has led to a variety of germination responses and dormancy types (Körner, 2003; Schwienbacher *et al*., 2011; Hoyle *et al*., 2015). The alpine habitat's different environmental gradients, such as those in temperature and water, which can be found within a few meters of one another (Graham *et al*., 2012) offer the perfect framework for evaluating changes in germination strategies in relation to the immediate environment. For example subalpine species have cooler germination temperatures than alpine species and are less dormant (Baskin and Baskin, 2014). In many arctic and alpine plant species fresh seeds germinate quickly even without any cold stratification (Sayers and Ward, 1966; Bell and Bliss, 1980). However, very high temperatures are required for arctic and alpine species to germinate, which could prohibit germination at an inappropriate time the fleeting arctic summer (Billings and Mooney, 1968).

Asteraceae (Compositae) is the largest and one of the most advanced Angiosperm families in plant kingdom. In this family, there are 1700 genera and 25,000-30,000 species found all over the world (Mandel *et al*., 2019). Asteraceae are cosmopolitan and has a wide distribution range. Species of this family are found in a variety of environment from tundra to tropical evergreen rainforests (Baskin and Baskin, 2014). Many species are used in the ornamental and medicinal purpose. For the purpose of food and vegetables, some species are commonly cultivated in field (Lawrence, 1973). This family includes many problematic invasive, weed species that produce numerous small and fertile seeds which have high seed dispersal range, rapid growth rate and greater reproductive efforts (Yuan and Wen, 2018).

Asteraceae seeds require cold stratification to crack the physiological dormancy and expand germination (Baskin and Baskin, 2002; Brandel, 2004). In Asteraceae family, the newly collected seeds are either non-dormant (ND) or have non-deep physiological dormancy (PD) (Baskin and Baskin, 2014). Different environmental elements like temperature, moisture, soil, radiation and humidity affected by climate change influence species diversity and composition by avoiding, slowing down or speeding up the germination process and thus resulting of the phenological shift (Walck *et al*., 2011). Moreover, at different water potentials germination responses are species specific (Fenner and Thompson, 2005; Baskin and Baskin, 2014).

Phylogenetically closely related species share identical seed-germination characters and not move too far from their perfect niche (Zhang *et al*., 2014). Within family or a genus, germination approaches may be a fixed evolutionary character, thus inhibiting interspecific differences in germination (Zhang *et al*., 2014; Carta *et al*., 2016). Species interact more greatly with near relatives than with faraway relatives (Cornwell and Ackerly, 2009).

1.2 Justification

Asteraceae is the largest plant family in the world. Funk *et al*., (2005) mentioned that the Asteraceae family is the most diverse plant family, occupying range of habitats from tropical evergreen rainforests to tundra. Hence, the family is considered to be a model system for evolutionary studies (Baskin and Baskin, 2014; Palazzesi *et al*., 2022). However, there have been few studies related to the dormancy and seed germination of the Asteraceae, in association with its ecology and bio geographical distribution (Baskin and Baskin, 2023).

Due to extremely restricted distribution of plants in high altitudinal regions, efforts about their germination behavior and ecology are limited (Liang *et al*., 2018). Most of the Asteraceae species have a tendency to become invasive, a feature acquired due to number of traits in the members of this plant family such as diaspore heteromorphism (Zhang *et al*., 2019), dispersal capacity and successful germination rates due to the presence of a crowned hairy or bristly pappus in their seeds (Hale *et al*., 2010) and also due to their rapid adaptive evolution (Datta *et al*., 2017). Likewise, members of the Asteraceae family show great diversity in all climatic and vegetation zones, including alpine and high Himalayan regions of Nepal. Furthermore, the seed germination of behavior of members of Asteraceae species may be influenced by the several factors such as, light and temperature gradients along different slopes and habitats (wet and dry).

Hence, this study was carried out to investigate the germination rate of seeds in laboratory conditions. Since seed germination may be affected by several abiotic components (Fenner and Thompson, 2005), this study was carried out to determine the germination patterns of six Asteraceae species from two contrasting precipitation regions (wet and dry). The results from these experiments give an idea about the optimal conditions required for the germination of the seeds of the investigated plant species. The results of this study also provide insights on how the changes in environmental conditions may affect seed germination of the mountain plants. Such insights are particularly important for understanding potential impacts of climate change on high mountain plant species of the Himalaya.

1.3 Hypothesis

Hypothesis of this study is:

- Species from dry habitat have higher stress tolerance than species from wet habitat.

1.4 Objectives

The general objective of the present study is

- To study the germination behavior of selected Asteraceae species from two regions with contrasting precipitation in the Himalaya.

The specific objectives of this study are

- To analyze variation in germination pattern of selected Asteraceae species from two regions with contrasting precipitation.
- To study changes in germination patterns of the selected Asteraceae species in response to environmental gradients.

1.5 Limitations

We had only one growth chamber available in laboratory. So, the effect of different temperatures (Low and high) on seed germination could not be run simultaneously. Also, due to the lack of meteorological station in the study area of Annapurna Base Camp (ABC), nearest meteorological station of Lumle (Latitude: 28.29654˚N, Longitude: 83.81791˚E) and 1738m asl was taken as reference point.

CHAPTER 2 LITERATURE REVIEW

2.1 Seed traits of species from contrasting habitats

From two different habitats wet and dry habitat revealed that seeds from species of wet habitat had significantly greater germination percentage than from dry habitat at lower water potential. At -0.25 MPa water potential 77±3% of the seeds germinated from wet habitat whereas 62± 3% germinated from dry habitat and -0.5MPa water potential 61± 4% seeds germinated from wet habitat and only 44±3% germinated from dry habitat (Ludewig *et al*., 2014).

Germination of two endemic *Rhaponticum* species (Asteraceae) such as *Rhaponticum bicknellii* and *Rhaponticum scariosum* in two different climatic zones showed that where *Rhaponticum scariosum* germinated at warmer temperature like warmer soil and air condition and *Rhaponticum bicknellii* seeds were adopt a drought avoiding germination action and after the snowmelt seeds were immediately germinate (Carasso *et al*., 2020). Seed germination of population of *Tragopogon pratensi* in two different habitats roadside verges and hayfields found that faster and greater germination percentage was found in roadside verge than in hayfield (Jorritsma-Wienk *et al*., 2007).

Seeds of seven *Stipia* species from cold and warm habitats found that germination reaction to water potential varied between the species but not among the habitats. Seeds from the cold-moist habitat were more tolerant of maximum temperature than the seeds from hot-dry habitats. There was no fair arrangement in response to water potential in relation to surrounding types noted by Zhang *et al*., (2020). Patterns of seed germination were not constant among the fellfield and snowbed environments. For interspecific differentiation no remarkable contrast in germination between fellfield and snowbed habitats at different temperature regime and cold stratification. In both fellfield and snowbed habitats, there was no difference between the germination rate and Mean Germination Time (MGT) (Shimono and Kudo, 2005).

Chen *et al*., (2013) studied the positive photoblastic germination response of four *Ficus* species from contrasting habitats in a seasonal tropical rainforest. Plant growth of gap demanding species like *Ficus hispida* and *Ficus racemosa* inhibited under low R:FR at

22°C /23°C but was unaffected over the R:FR ratio gradient at 25°C/35°C and germination percentage of shade tolerant species *Ficus altissima* and *Ficus auriculata* were not inhibited from all treatments along the full light gradient. *Sarcocaria fruticosa*, a high marsh species, germinated in dark with enhanced germination after being exposed to saline environment for a long time whereas a medium marsh hybrid taxon called *Sarcocaria perennis* × *fruticosa* displayed intermediate reactions. It shared *Sarcocaia fruticosa* ability to germinate at a high rate in hyper saline environments as well as *Sarcocaria perennis* lack of germination in the dark and of fast germination (Redondo *et al*., 2004).

In terms of germination behavior, calcareous and siliceous grasslands dramatically differ from one another, with slow, mostly overwinter germination and strong germination under all conditions, respectively. High overwinter germination species are primarily found in heathlands and have an arctic-alpine range. While species with a generalized low or high germinability tend to live in grasslands or lack a specialized microhabitat (Tudela-Isanta *et al.*, 2017).

2.2 Asteraceae of Nepal Himalaya

Asteraceae (or Compositae, the Sunflower, Daisy and the Aster family), is the largest and advanced family of angiosperms. In this family, there are 1700 genera and 25,000-30,000 species found all over the world (Mandel *et al*., 2019). In the context of Nepal, a total 128 genera and 414 species have been documented (Shrestha *et al*., 2022).

Chhetri and Bhattarai (2013) organized a floristic study of the Upper Manaslu Conservation Area (MCA), Central Nepal and reported that the Asteraceae was the dominant plant family with 12 genera and 20 species including *Anaphalis* sp., *Artemisia* sp., *Aster* sp., *Cremanthodium* sp., *Gerbera nivea*, *Inula hookeri*, *Ligularia fischeri* and *Senecio wallichi*.

Chalise *et al*., (2019) reported that Asteraceae was the dominant family with 40 species belonging to 21 genera while doing floristic study in Gyasumbdo Valley, Lower Manang, Central Nepal and those species were *Anaphalis* sp, *Aster* sp, *Circium* sp, *Cremanthodium* sp, *Dubyaea hispida*, *Saussurea* sp, *Synotis* sp, *Taraxacum* sp, *Inula hookeri* and *Ligularia fischeri*.

The Asteraceae species with the most promising natural antioxidant qualities for usage in medicine, cosmetics, and food are identified. Astringents, anti-inflammatory medications, diaphoretics, nerve tonics, laxatives, wound healing products, blood flow abnormalities, headaches, pains, flatulence, dysentery, haemorrhoids, ulcers, and cachexia-causing illnesses are all members of this family (Sharma *et al*., 2022).

The plant *Pteronia onobromoides* (Asteraceae) leaves where used in cultural and commercial benefits. The leaves powdered were mixed with fat and apply in skin for medical/ cosmetic purposes (Hulley *et al*., 2010).

2.3 Seed size and mass of Asteraceae species

Seed size and seed morphology separately influence germination and seedling growths (Zhang, 1993). For seed mass, small seeds may gather dynamic influence due to a higher dispersal capacity that enhance the probability of passing favorable microsite but the large seeds may allow rise to exceeding competitors due to higher germination percentage, lower light concern for germination and higher emergence from deeper depth (Jankowska-Blaszczuk and Daws, 2007).

Germination of small seeded species needed light but the large and intermediate sized seeds expose greater germination percentage and lower mean times to germinate than small seeded species (Galindez *et a*l., 2009). Germination of large seeded species requires less light in comparison to small seeded species (Milberg *et al*., 2000). Large seeds were produced by *Flourensia cernua* because there was a higher bearing of filled seeds (Valencia-Diaz and Montana, 2005). It also indicates that seed quality was affected by yearly variation in the winter temperature of the maternal environment while seed size was also affected by annual rainfall.

Seed dimorphism occurs when a single plant develops two seed types with distinct form and/or behavior. Van Molken *et al*., (2005) observed that larger seeds produce higher germination percentages and seed morphology had no direct effect on the germination process. Seed size controlled the germination behavior rather than by seed morphology.

2.4 Seed germination patterns of Asteraceae species

Li *et al*., (2007) reported that *Echinacea purpurea* seeds obtained higher germination percentage when treated with gibberellic acid (GA_3) and ethephon. Also prechilling treatment to the seeds elevated their germination rate and percentage in both dark and light periods and germination was also improved when the seed coat was removed. Seeds of *Artemisia* species such as *Artemisia wudanica*, *Artemisia halodendron*, *Artemisia sieversiana* and *Artemisia scoparia* obtained higher germination percentage when they were treated in chilled and long term dry storage. Out of four species, *A. scoparia* attained greater germination percentage when the seeds were store at dry condition rather than chilled (Li *et al*., 2012).

According to Martinez-Garcia *et al*., (2012), the seeds of *Senecio coincyi* showed 90–100% germination rates at 15°C to 30°C and germination was not affected by light condition. *Brachycome muelleri* seeds germinated when dormancy was subdued by gibberellic acid (GA_3) under a maximum temperature was 20°C as stated by Jusaitis *et al*., (2004).

Moreover, the seeds of *Polymnia canadensis* attained greater germination percentage after cold stratification and germination was higher in light than in dark condition as observed by Bender *et al*., (2003). Freshly collected seeds of *Silybum marianum* did not germinate at any temperature and light conditions but after treating the seeds with GA_3 and light increased germination percentage in dark at any range of temperature as noted by Monemizadeh *et al*., (2021).

Bidens pilosa attained significantly greater germination percentage when seeds were treated in effect of GA_3, subjected to cold stratification and were dry stored reported by Zhang *et al*., (2019).

2.4.1 Dormancy

Seed dormancy can directly prevent/promote the germination or can make the seeds postpone their germination even when the environment is favorable for germination (Baskin and Baskin, 2004). In Asteraceae family, the newly collected seeds have either non-dormant (ND) or have non-deep physiological dormancy (PD) (Baskin and Baskin, 2014). Freshly harvested seeds of some species have non-dormant dormancy and they can germinate over a large scale within the specified time (Baskin and Baskin 2014; Kildisheva *et al*., 2020).

Bender *et al*., (2003) found that seeds of *Polymnia canadensis* (from the family Asteraceae) carry physiological dormancy but generated a higher seed germination percentage after they were subjected to cold stratification. Seeds of *Rhaponticum bicknellii* were also physiologically dormant (Carasso *et al*., 2020).

Similarly, two types of non-deep physiological dormancy were present in *Silybum marianum* (Asteraceae) during seed development as stated by Monemizadeh *et al*., (2021). Furthermore, Mattana *et al*., (2009) reported that two types of non-deep physiological dormancy were also found in *Lamyropsis microcephala*.

Li *et al*., (2012) reported that seeds of *Artemisia scoparia* had innate dormancy which was broken by chilling and long term dry storage. Likewise, the seeds from both the central and marginal seeds of *Bidens pilosa* showed non-deep physiological dormancy which was broken when the seeds were treated with GA_3, subjected to cold stratification and kept for dry storage as mentioned by Zhang *et al*., (2019).

2.4.2 Light requirements

One of the most important regulatory environmental signals in seed germination is light (Gutterman, 1993). Seeds of Neotropical pioneer small seeded species need light for germination in comparison to other larger-seeded species (Pearson *et al*., 2002).

Within both light and dark periods, prechilling treatment can significantly increase the germination rate and percentage of the seeds. Seeds prechilled in the dark had a lower germination percentage and germination rate compared to seeds prechilled in the light (Li *et al*., 2007). Effect of light on seed germination of *Echinacea purpurea* was dynamic, either encouraging seed germination (Smith-Jochum and Albrecht, 1987) or having no impact (Wartidiningsih and Geneve, 1994).

Seeds of *Artemisia sphaerocephala* (Asteraceae) germinate in light but not in dark (Huang and Gutterman, 1999). The seeds of *Artemisia sphaerocephala* are delicate to far –red light and germination was enhanced than in the dark (Koller *et al*., 1964). Seeds of four *Artemisia* species such as *A. wudanica*, *A. halodendron*, *A. sieversiana* and *A. scoparia* obtained greater germination percentage under light regimes than in the dark. Moreover, dark had no effect on the seed germination of two semi-shrubs *A. wudanica* and *A. halodendron* but it discouraged germination in the seeds of the annuals species such as *A. sieversiana* and *A. scoparia* (Li *et al*., 2012).

Milberg *et al*., (2000) reported that germination and light requirement coexist with seed mass. Also, Jankowska-Blaszczuk and Daws, (2007) conclude that in temperate forest, small-seeded herbs needed more light for germination and due to competition from the photoblastic species, greater standard of light (R: FR) is required for herbs with small seeds. In case of light quality, suitable microsites are required for the germination of small seeded species.

Kumar and Sharma (2012) found that the seeds of *Tagetes minuta* don't demand light. According to Afolayan *et al*., (1997), the seeds of *Helichrysum aureonitens* required light and suitable temperature for germination. Further, the seeds of *Helichrysum apiculatum* revealed higher germination percentage when exposed to light than in the dark.

Seeds of Mexican Sunflower, *Tithonia rotundifolia*, exhibited higher germination rates in dark than in the light because of non-photoblastic nature as mentioned by Wen, (2015). However, *Oritrophium peruvianum* seeds favor light for germination than in the dark (Ulian *et al*., 2013).

2.4.3 Effects of temperature

Afolayan *et al*., (1997) reported that, *Helichrysum aureonitens* seeds germinated higher at temperature between 25°C and 30°C than 15 and 35°C. The suitable temperature range for seed germination was 25-30°C under the incidence of light. Similarly, only 1% germination was obtained at 35°C under light regimes. Greater germination percentage was obtained within the temperature range of 15-35°C. However, between the temperature range of 10°C and 40°C germination percentage declined and between 5°C and 45°C seeds didn't germinate at all in the seeds of the studied species; *Cichorium intybus*, *Cynara scolymus*, *Echinacea purpurea*, *Achillea millefolium* and *Matricaria aurea (*Zarghani *et al*., 2014).

Seeds of *Stevia rebaudiana*, *Salvia sclarea* and *Tagetes minuta* were treated within a range of two factors light periods and under different temperature regimes, (room temperature, 20°C, and 10°C). In all the three species maximum germination was recorded when seeds were placed in room temperature for two days and placed in continuous light at 20°C (Kumar and Sharma, 2012).

According to Huang and Gutterman (1999), the most favorable temperature for germination of seeds of *Artemisia sphaerocephala* was 25°C, after rainfall. From the results it was observed that the germination was slow and germination percentage was low at 10°C and 30°C. Also, the seeds did not germinate even at 5°C. According to Li *et al*., (2007), seeds of *Echinacea purpurea* showed greater germination percentage when they were treated with GA_3 at constant temperature 20°C and alternating temperature range of 20°C to 30°C. Hence, no significant difference was obtained for germination percentage at both constant and alternating temperature regimes.

Likewise, Wen (2015) observed the seeds of *Tithonia rotundifolia* that exhibited to have maximum germination at fluctuating temperatures of 18/28°C. However, at 35°C, the germination percentage of the seeds were noted to be between 20% and 40%. It was observed that the suitable temperature regime for seed germination is between 15°C and 30°C. Furthermore, germination was found to be higher in darkness than in light periods between 10°C and 40°C showing the non-photoblastic nature of the seeds.

According to Martinez-Garcia *et al*, (2012), the seeds of *Senecio coincyi* attained higher germination percentage 90–100% between the temperature regimes of 15°C and 30°C. However, the germination percentage declined at 10°C (19%). Similarly, Ulian *et al*., (2013) reported that the seeds of *Oritrophium peruvianum* showed higher germination percentage at an alternating temperature regime (average low temperature 16°C and average high temperature 24°C) when compared to the constant temperature periods between 25°C and 26°C.

2.4.4 Effect of water stress

Accessibility to water decreases seed germination of species. Neckar *et al*., (2008) worked on the seeds of Asteraceae family under water deficit condition by using PEG 6000 (Polyethylene Glycol) solution having different concentrations and different water potentials (0, -0.05, -0.1, and -0.5). From the results, the lowest value of seed germination was obtained at -0.5 MPa water potential.

According to Wen (2015), germination percentage of *Tithonia rotundifolia* was significantly reduced at -0.3 MPa water potential when compared with the control groups. Seeds with equal water potential between -0.5 MPa and -1 MPa revealed to have lower germination when treated with PEG 8000 as compared to sodium chloride (NaCl) solution. The germination percentage reduced to 20% at -0.6 MPa water potential when treated with PEG or at -1 MPa when treated with NaCl.

Toscano *et al*., (2017) reported that, 90% germination was obtained from the seeds of ornamental Sunflowers (*Helianthus annuus*) at no water stress (control) when tested between all cultivators. Among the three types of Hybrid Sunflowers ('Hadar,' 'Pazit,' and 'Zohar') Hadar and Pazit performed better at -0.45 MPa water potential with a germination percentage of ≥80%. However, 23% germination was obtained at -0.6 MPa water potential. In case of Zohar, 80% germination was obtained at -0.6 MPa water potential and the seeds also displayed germination at -0.75 MPa water potential.

According to Yuan and Wen (2018), three species such as *Crassocephalum crepidioides, Conyza canadensis* and *Ageratum conyzoides* attained higher germination percentage i.e. 40%, 50% and 65% at -0.6MPa water potential while treating with PEG 8000 and NaCl with uniform water potential gradient between -0.05 and -1Mpa. *Crassocephalum crepidioides* and *Ageratum conyzoides* were more sensitive to PEG than NaCl while *Conyza canadensis* was more sensitive to NaCl.

Ping *et al*., (2011) reported that when using PEG 6000 seeds of *Chromolaena odorata* was germinated 78, 74, 68, 64 and 63% at 0, -0.1, -0.2,-0.3 and -0.4 MPa water potential. At -0.5, -0.6 and -0.7 MPa water potential only 29, 15 and 5% of seeds germinated. No germination obtaining at -0.8 -0.9, and -1.0 MPa water potential. Greater germinations were obtaining at osmotic potentials between 0 and -0.4 MPa. Seeds of *Centaurea* *eriophora* treated in PEG 6000 with water potentials 0, -0.03, -0.08, -0.19, -0.47, -1.2 and -2.45 MPa. 85% germination was occurred at 0MPa and lowest germination was found at -1.2 MPa i.e. 6.66% and germination was totally inhibited at -2.45MPa water potential (Bouker *et al*., 2022).

2.5 Research Gap

There are a few studies on the germination of high Himalayan plants and little knowledge about it. Earlier research have mainly dedicated to germination, emergence and implication for invasiveness in *Taraxacum* species (Letchamo and Gosselin, 1996; Luo and Cardina, 2012). Moreover, there is absence of details on the effect of environmental stress, growth of these wild species and climate change which can provide the important information about their adaptation, invasiveness and global warming. There was no record of germination behavior of the species such as *Cremanthodium arnicoides*, *Cremanthodium reniforme*, *Synotis alata*, *Synotis penninervis*, *Dubyaea hispida* and *Taraxacum eriopodum*. There is lacking of studies about germination of species in two contrasting precipitation habitat.

CHAPTER 3 MATERIALS AND METHODS

3.1 Species Characteristics

Six species of the Asteraceae *Cremanthodium reniforme*, *Cremanthodium arnicoides*, *Synotis alata*, *Synotis penninervis*, *Dubyaea hispida* and *Taraxacum eriopodum* were selected for the study (Table 1). Those species were selected on the basis of phylogenetic closeness of the six species *Cremanthodium reniforme*, *Cremanthodium arnicoides*, *Synotis alata* and *Synotis penninervis* (Fig. 1) belong to same tribe Senecioneae and same subfamily Asteroideae (Fu *et al*., 2016) and the species *Dubyaea hispida* and *Taraxacum eriopodum* (Fig. 1) belong to subfamily Cichorioideae which is closely related with subfamily Asteroideae (Fu *et al*., 2016).

Table 1: Description of the study species

Species	Common name	Distribution	Habit and habitat
Cremanthodium reniforme (DC.) Benth.	Tractor Seat plant	Central and eastern Himalaya to China. Western, Central and Eastern Nepal (3800-4900 m) (Shrestha *et al*., 2022).	Deciduous perennial herb with kidney shaped, moist area
Dubyea hispida DC.	Bristly Dubyaea	Himalaya to China and north Myanmar Western, Central and Eastern Nepal (2700-4300 m (Shrestha *et al*., 2022).	perennial herb, moist area

Synotis alata (Wall. ex DC.) C.Jeffrey and Y.L.Chen	Winged Senecio	Himalaya to China and Myanmar Western, Central and Eastern Nepal (1500-4400 m) (Shrestha *et al*., 2022).	perennial herb, moist area
Cremanthodium arnicoides (DC. ex Royle) R.D.Good	Arnica Himalayan Sunflower	Himalaya to south Tibet, Western and Central Nepal (3100-4900 m (Shrestha *et al*., 2022).	perennial herb, dry area
Synotis penninervis (H.Koyama) T.J.Tong, M.Tang, C.Ren and Q.E.Yang		Himalaya to south Tibet. Western and Central Nepal at the range of 2800-4500m (Shrestha *et al*., 2022).	perennial herb, dry area
Taraxacum eriopodum (D.Don) DC.		Himalaya (Kashmir to Bhutan), Assam, Tibet, West China. Western Central and Eastern Nepal (2100-4600m (Shrestha *et al*., 2022).	Perennial herb, dry area

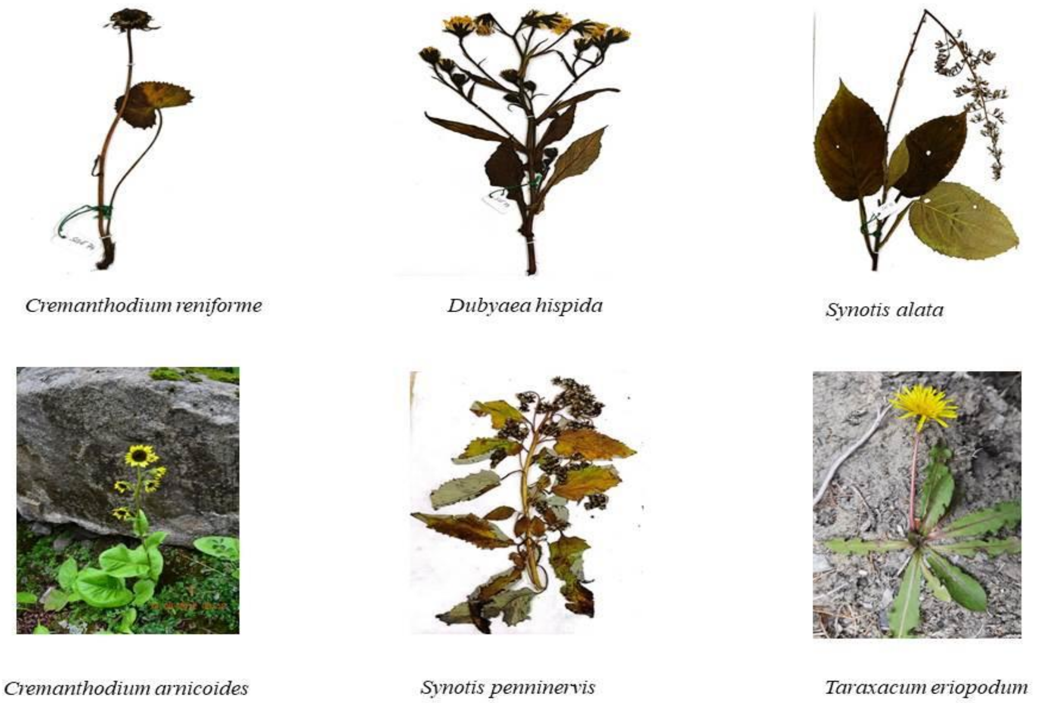

Figure 1: Study species

3.2 Seed collection site

Seed of *Synotis alata*, *Dubyaea hispida* and *Cremanthodium reniforme* were collected from Deurali to Annapurna Base Camp (ABC) (Fig. 2C) which is located in Kaski district of Gandaki province. The area lies on the southern declivity of Annapurna mountain range. The dominant tree species in the region are *Betula utilis*, *Abies spectabilis*, *Salix* spp., *Sorubus* spp., *Prunus* spp. and *Juniperus* spp. Seeds of *Cremanthodium arnicoides*, *Synotis penninervis* and *Taraxacum eriopodum* were collected from Manang district of Gandaki province (Fig. 2D). The seed collection sites in Manang district is located at the broad valley of Marshyangdi river to the north of the Annapurna mountain range. It is also included in Trans-Himalayan biogeographic region (Dobremez, 1976) and has vegetation similar to Tibetan Plateau (Chaudhary, 1998). Dominant tree species in the region are *Pinus wallichiana, Betula utilis, Abies spectabilis* and *Juniperus* spp. Both seed collection sites lies in Annapurna Conservation Area (ACA) and it covers 7629 km^2 which is the largest protected area of Nepal (DNPWC, 2016).

Annapurna Base Camp is one of the moist regions of Nepal. At the year of 1991-1994, the precipitation of Lumle was over 5100 mm $year^{-1}$, Ghandruk 2900mm $year^{-1}$ and Machapuchre Base Camp was 1664 mm $year^{-1}$ (Braun *et al*., 1998) while the precipitation of Manang was 442 mm $year^{-1}$. Manang valley is one of the driest regions of Nepal Himalaya, it is situated in the Trans-Himalayan region and the rain shadow affects the surrounding Himalayas which make it dry (Karki et al., 2015).

Table 2: Seed collection for study species

Study species	Location	Collection date	Latitude (˚N)	Longitude (˚E)	Elevation (m)
Cremanthodium reniforme (Wall.ex DC.) Benth.	Kaski, above Macchapuchre Base Camp	25th September, 2022	28.52838	83.89433	3960
Dubyaea hispida DC.	Kaski, above Macchapuchre Base Camp	24th September, 2022	28.52745	83.90272	3761
Synotis alata (Wall. ex DC.) C.Jeffrey & Y.L.Chen	Kaski, below Macchapuchre Base Camp	23rd September, 2022	28.51847	83.90775	3504
Cremanthodium arnicoides (DC.ex Royle) R.D. Good	Manang, Yakkharka	17th October, 2022	28.72911	83.970927	4120
Synotis penninervis (H.Koyama) T.J.Tong, M.Tang, C.Ren & Q.E.Yang	Manang, Thorang bridge	18th October, 2022	28.76298	83.96676	4404
Taraxacum eriopodum (D.Don) DC.	Manang, Humde	22 May, 2022	28.638187	84.089815	3387

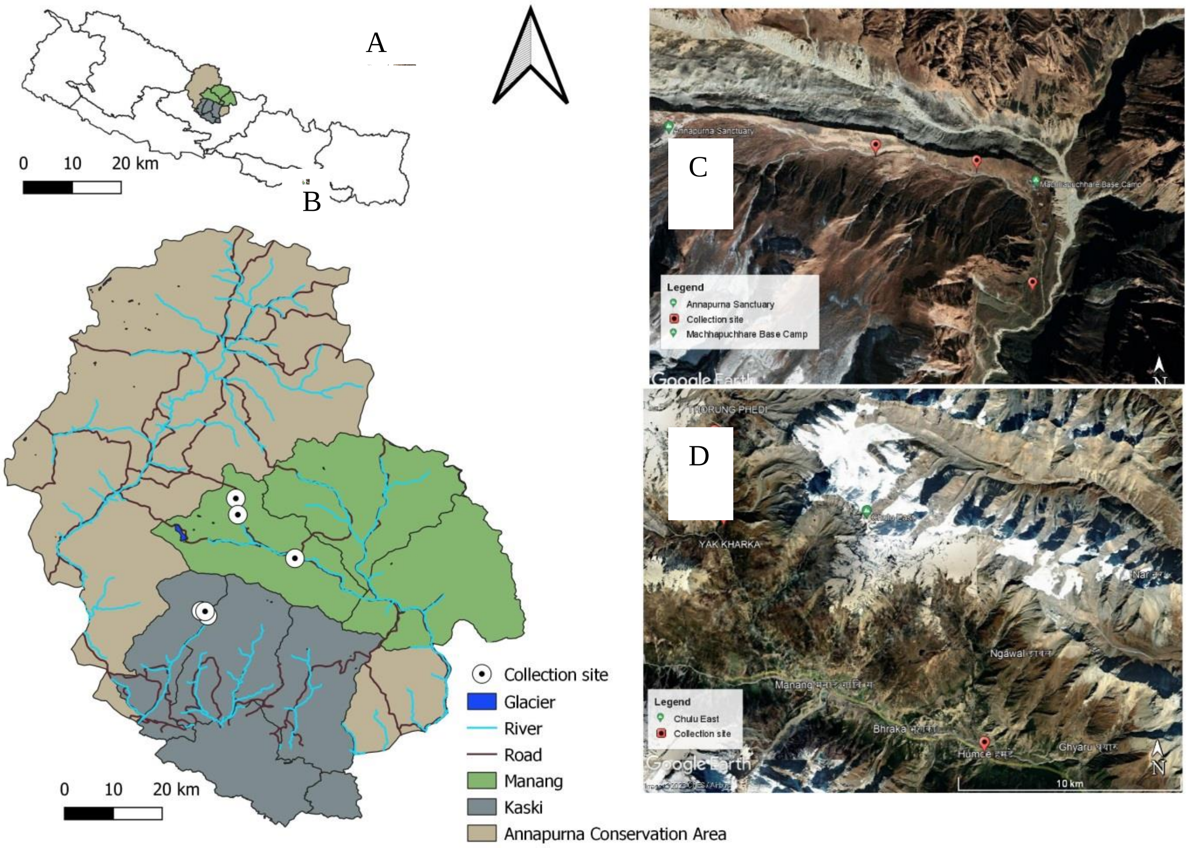

Figure 2 A) Map of Nepal B) Annapurna Conservation Area showing Kaski and Manang C) Seed collection site in Annapurna Base Camp D) Seed collection site in Manang

The past 11 years (2012-2022) climatic data was recorded from Lumle were annual precipitation of was 5254mm. The maximum precipitation was recorded 1511mm in July and minimum precipitation 4mm was recorded in November. The maximum temperature was recorded 24˚C in August and minimum temperature at 4˚C in January (Fig. 3A) (Department of Hydrology and Meteorology).

Weather data collected at Humde meteorological station (Manang) (Latitude: 28.638197 ˚N, Longitude: 84.089815 ˚E and 3387m asl) for eight years (2015-2022) was taken as a reference.

Annual precipitation of Humde was 452.03mm. The maximum precipitation was recorded 74.75mm in July and minimum precipitation 1.54mm was recorded in November. The maximum temperature was recorded at 19˚C in August and minimum temperature at -10˚C was in January (Fig.3B) (Department of Hydrology and Meteorology).

3.3 Seed collection and Storage

Seeds were collected during September 2022 from Deurali to Annapurna Base Camp (ABC) and October 2022 from Manang (Table.2). Seeds of all species were collected from the mature and healthy plants. Only fully ripened seeds were collected. All seeds were collected when natural dispersal began (Fig.4). After collection, seeds were kept in muslin cloth and transported to the Ecology laboratory of Central Department of Botany, Tribhuvan University, Kirtipur. Seeds were air dried at room temperature for 7 days. An immature, damaged and infected seeds were discarded. Separated healthy, matured and intact seeds were placed in air tight plastic bottle and stored at 4˚C in refrigerator until needed (Chauhan and Johnson 2008; Baskin and Baskin 2014). To absorb the moisture of seeds silica gel (particle size: 6-20 mesh) was used.

3.4 Seed mass

For seed mass, lot of 100 seeds for *Synotis alata*, *Synotis penninervis* and *Taraxacum eriopodum* and lot of 50 seeds for *Dubyaea hispida*, *Cremanthodium reniforme* and *Cremanthodium arnicoides* were taken according to their seed size. The seeds were oven dried at 60℃ for 72 h. Three replicates for each species were taken. Mean seed mass was measured with the help of digital weighing balance (0.0001g) Model (MG214Ai) (Baskin and Baskin, 2014).

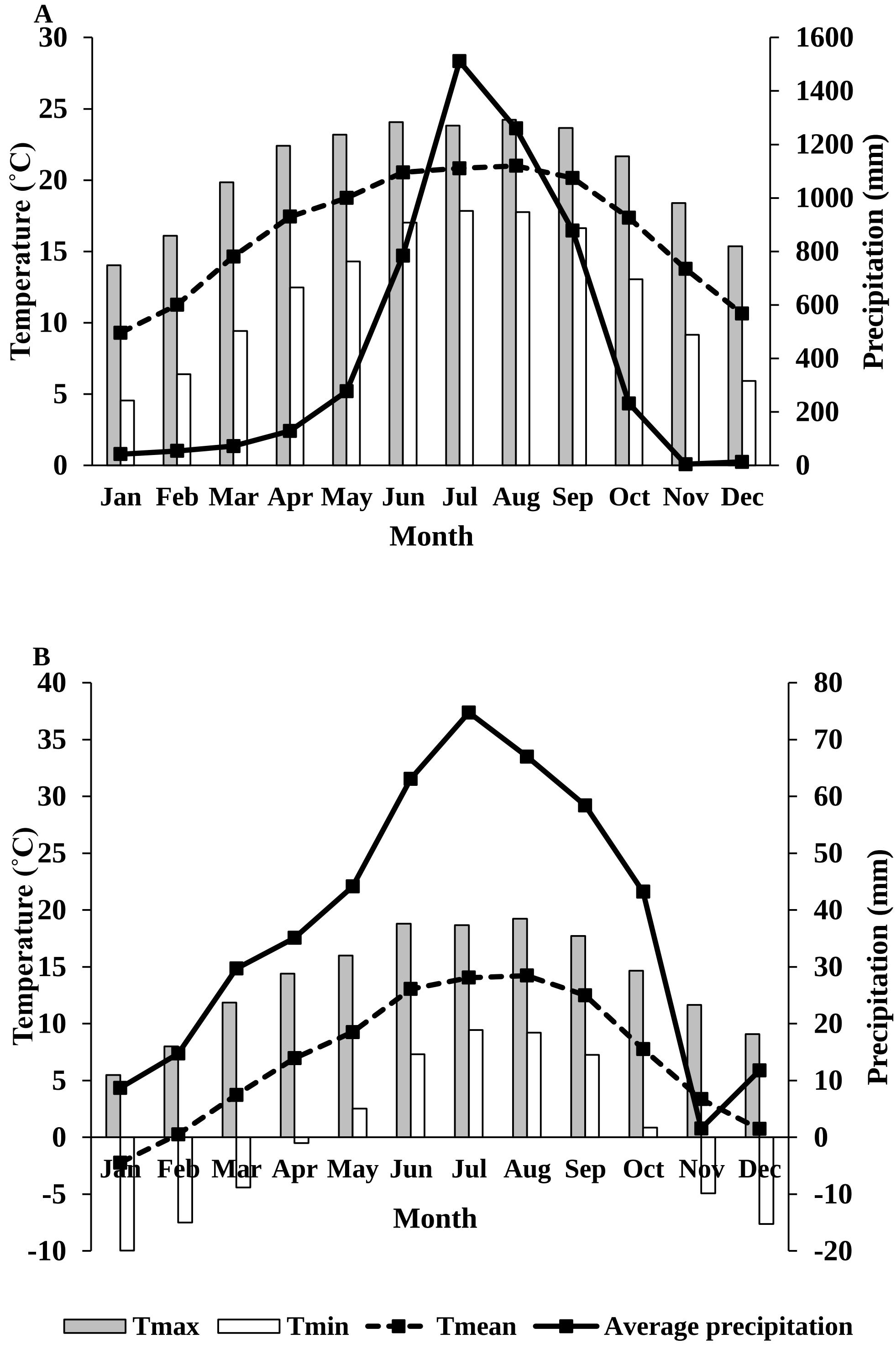

Figure 3: (A) Mean of maximum and minimum temperature as well as precipitation recorded between 2012-2022 at Lumle station of Kaski, Nepal (B) Mean of maximum and minimum temperature as well as precipitation recorded between 2015-2022 at Humde station of Manang, Nepal (Department of Hydrology and Meteorology)

Seed collection   Seed cleaned   Seed storage

Figure 4: Seed collection and storage

3.5 Seed Size

For seed size, 20 seeds were taken for each species and measured the length and breadth (Fig.5). Seed size was measured by using stereomicroscope (Model: STMLAB-T LED) BEL ENGINEERING SRL ITALIA and “Wave Image” software and camera (Model: EUREKAM 20 PLUS).

3.6 Preliminary germination test

The germination procedure was carried out immediately so that the storage process would not change the germination behavior of the seeds (Baskin and Baskin, 2014). Hence, the preliminary tests were carried out immediately after a week (4-7 days). In the process all seeds began to germinate after incubation of 4-5 days and most of them germinated in 14 days.

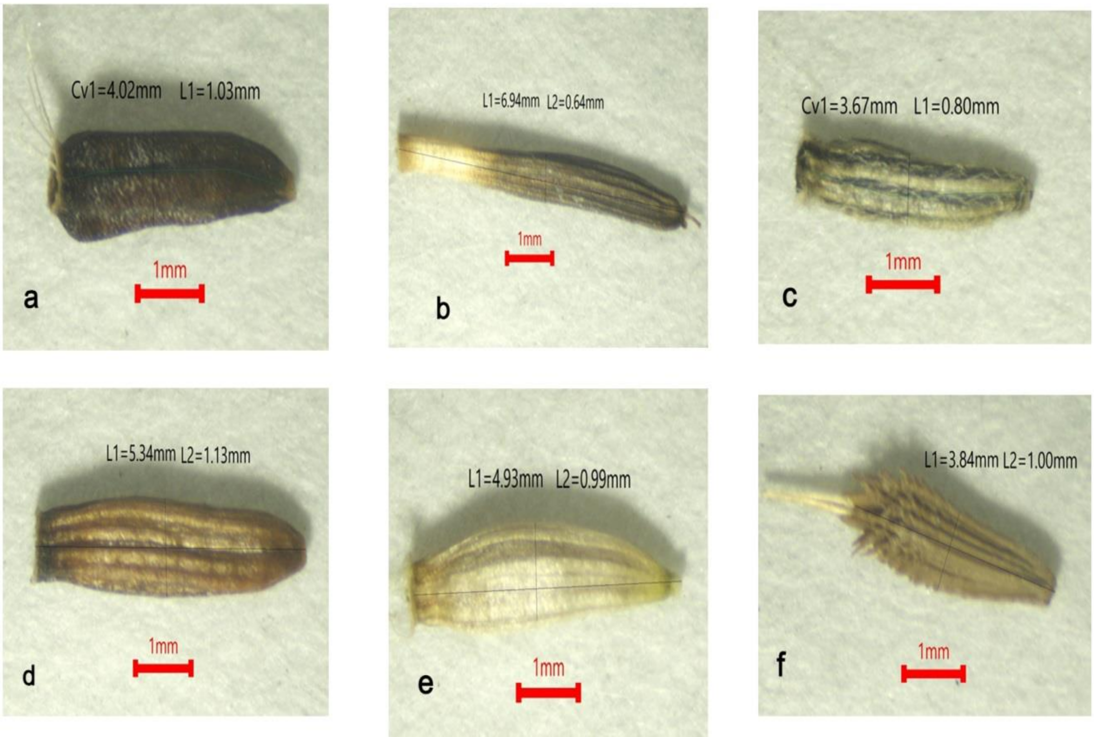

Figure 5: Seed size measurement of studied species a) *Cremanthodium reniforme* b) *Dubyaea hispida* c) *Synotis alata* d) *Cremanthodium arnicoides* e) *Synotis pennincrvis* f) *Taraxacum eriopodum*

3.7 Germination experiment

Seeds were tested for dormancy in preliminary germination experiment within 7-10 days after collection. After the preliminary test, full cycle of germination experiments were run as seeds germinated in 4-5 days of incubation. Germination experiment was done in different environmental conditions such as light, temperatures and different water stress conditions. In germination experiment 30 mature and uniform sized seeds were equally distributed in 9-cm diameter Petri dish with a 2 layer of filter papers which were then moistened either with 3-ml of distilled water or 3-ml of different concentration of Polyethylene Glycol (PEG 6000) solution (Fig. 6). There were five replications for each treatment; therefore for each treatment 150 seeds of each species were used. The Petri dishes were sealed with parafilm and incubated inside the growth chamber (GC-300TLH, Jeio Tech, Korea) for 14 days. Light intensity was maintained at 3000 lux and relative humidity at 70%. Other environmental conditions maintained inside the chamber has been described below. Radicle emergence is considered main criteria for seed germination (Chauhan and Johnson, 2009). Seed germination was evaluated daily for 14 days (Zarghani *et al*., 2014). For complete dark treatment germination was observed in 14^{th} day.

3.7.1 Effect of Temperature and light

The effect of temperature on germination was determined where seeds were incubated in growth chamber and maintaining two alternating temperature regimes 25/15°C and 30/20 °C simulating day/night conditions. The higher temperature (30/20°C) (Chauhan and Johnson, 2009) and lower temperature (25/15°C) was maintained with the 12-h photoperiod (white fluorescent light) with the intensity of 3000 lux. For continuous dark treatment Petri dishes were wrapped by two-layered aluminum foil and placed in growth chamber. Germination was recorded on14^{th} day of seed incubation.

3.7.2 Effect of Water Stress

Polyethylene glycol (PEG 6000) solution was used to know the effect of water stress on the germination of species. In order to prepare the PEG solution, 296 g of PEG 6000 was dissolved in distilled water to prepare 1 liter of PEG solution of -1MPa (Michel and Kaufmann, 1973). This solution was then diluted to prepare the PEG solution of other concentrations such as -0.1, -0.25, -0.5, -0.75 and -1 MPa. In growth chamber maintaining day/night temperature, whereas minimum temperature (25/15°C) and maximum temperature (30/20°C) with the intensity of 3000 lux. Those seeds which did not germinate at high concentration of PEG solutions -0.75 and -1MPa were rinsed with distilled water and added 3ml distilled water and placed inside the growth chamber again. The numbers of germinated seeds were counted after 7 days.

Figure 6: Different stages of seed germination experiment

3.8 Data analysis

3.8.1 Germination pattern

Germination percentage, Timson's Index and Mean germination time were calculated using following formulae (Baskin and Baskin, 2014).

$$\text{Germination \%} = \frac{\text{Germinated } seed}{Total\ seed} \times 100\%$$

Timson's Index measures germination rate and was calculated as

$$\text{Timson's Index} = \sum_{i=1}^{k} Gi$$

where, Gi = cumulative daily germination percentage in time interval i and k is total number of intervals

Mean germination time (days) measure of the time to have maximum germination and was calculated using following formula:

Mean germination time = $\sum_{i=1}^{k} niti / \sum_{i=1}^{k} ni$

Where, ti = time from start of experiment (day)to the 1st observation

ni = number of seeds germinated at time (not the cumulative number)

k = last time of germination

3.8.2 Statistical analysis

For statistical data analyses, prior to the parametric test, the data were checked for normality and homogeneity of variance. Fractional values were subjected to square root transformation first, and then degree arcsine transformation. An independent sample t-test was used to examine the impact of light, dark and temperature within each species and between species, an Analysis of Variance (ANOVA) with a Tukey post-hoc test was used. A one-way ANOVA, tukey post-hoc test was conducted to examine the impact of light, dark and temperature among species. To analyze the water stress and species two-way ANOVA was conducted. All the analysis were done using Microsoft excel 2010 and IBM SPSS Statistics 25 (IBM Corp, 2017)

CHAPTER 4 RESULTS

4.1 Seed mass and seed size

The highest seed mass was found in *Cremanthodium arnicoides* from dry habitat and *Cremanthodium reniforme* from wet habitat (Table.3). Lowest seed mass was found nearly similar in *Synotis alata* and *Taraxacum eriopodum* from both wet and dry habitat. Out of six species the largest seed size was found in *Dubyaea hispida* from wet habitat and *Cremanthodium arnicoides* from dry habitat.

. **Table 3:** Mean (± SD) seed mass of study species (n=3)

Habitats	Species	Seed Mass (mg/seed)	Length (mm)	Breadth (mm)
Wet habitat	*Cremanthodium reniforme*	$1.47 \pm 7.29 \times 10^{-5}$	3.33 ± 0.225^{C}	0.91 ± 0.092^{a}
	Dubyaea hispida	$0.874 \pm 3.48 \times 10^{-5}$	7.93 ± 0.449^{A}	0.75 ± 0.076^{b}
	Synotis alata	$0.61 \pm 0.8 \times 10^{-5}$	2.82 ± 0.197^{D}	0.65 ± 0.065^{d}
Dry habitat	*Cremanthodium arnicoides*	$2.92 \pm 5.67 \times 10^{-5}$	3.88 ± 0.392^{B}	0.88 ± 0.089^{ac}
	Synotis penninervis	$1.11 \pm 12.1 \times 10^{-5}$	2.85 ± 0.275^{D}	0.76 ± 0.062^{c}
	Taraxacum eriopodum	$0.51 \pm 3.66 \times 10^{-5}$	2.36 ± 0.207^{E}	0.75 ± 0.117^{b}

Capital letter represents significant difference ($p \leq 0.05$) among the length of the species. Small letter represents significant difference ($p \leq 0.05$) among the breadth of the species.

4.2 Germination pattern

4.2.1 Effect of light

The germination percentage of *Synotis penninervis* (93% in photoperiod and 94.67% in dark) and *Cremanthodium arnicoides* (97% in photoperiod and 96.67% in dark) were significantly greater than those of other species at low temperatures (25/15˚C) (Fig. 7A). As from wet region germination of *Synotis alata* was significantly higher as compared with *Cremanthodium reniforme* and *Dubyaea hispida*. However, from the (Fig.7A) it is evident that the species from dry regions showed almost equal Germination Percentage (%) under both dark and photoperiod (control sets), i.e. *Cremanthodium arnicoides* (97%), *Synotis penninervis* (93%), and *Taraxacum eriopodum* (73%). From moist region *Cremanthodium reniforme* and *Dubyaea hispida* both had roughly comparable percentage of germination in the dark and during the photoperiod, whereas *Synotis alata* had much higher rates (40% in the dark and 79% during the photoperiod). In wet region, no significant difference was found between *Cremanthodium reniforme* and *Dubyaea hispida* but significant difference was found in *Synotia alata* at continuous dark. At photoperiod, no significant difference was found in *Cremanthodium reniforme* and *Synotis alata* but difference was found in *Dubyaea hispida*. In dry region, no significant difference was found in *Cremanthodium arnicoides* and *Synotis penninervis* in both dark and photoperiod but significant difference was found in *Taraxacum eriopodum*.

But a contrasting difference in the Germination Percentage (%) from temperature experiments was seen for *Synotis* species from both wet and dry regions. At higher temperature (30/20˚C), the Germination Percentage (%) for *Synotis alata* (92%), from wet region, was found to be significantly higher than the Germination Percentage (%) for *Synotis penninervis* (68%) from the dry region (Fig.7B). These two species were significantly different in dark and photoperiod conditions. Other species like *Taraxacum eriopodum* and *Cremanthodium reniforme* had about comparable germination rates in both dark and light conditions. *Dubyaea hispida* and *Cremanthodium arnicoides* also had nearly equal germination rates. Species from wet region germinated high at high temperature (30/20˚C) than low temperature (25/15˚C). In wet region, no significant differences was found in dark but in photoperiod significant difference was found between *Synotis alata* and *Dubyaea hispida* and *Synotis alata* and *Cremanthodium reniforme*. In dry region, significant difference was found in dark but in photoperiod no significant difference was found between species.

4.2.2 Effect of temperature

At low temperature (25/15˚C), there was significant difference found in germination percentage at wet and dry regions (Fig. 8a). Among species significant differences was found between *Dubyaea hispida* and *Synotis alata* from wet region but in dry region no significant differences was found between *Cremanthodium arnicoides* and *Synotis* *penninervis* but significant differences was found in *Taraxacum eriopodum*. Similarly significant difference was found in Mean Germination Time (MGT) at the wet and dry region (Fig. 8b). For Timson's Index (TI) no significant difference found between *Cremanthodium reniforme* and *Dubyaea hispida* and differences found in *Synotis alata* in wet region but no difference found in dry region (Fig. 8c).

At high temperature, significant difference in the germination percentage was found in wet region but no difference found in dry region in germination percentage (Fig. 8a). Similarly, significant differences were found in MGT in both wet and dry region (Fig. 8b). Also, no significant differences were found between *Dubyaea hispida* and *Synotis alata* in wet region and dry region significant differences were found for the values of Timson's Index (Fig. 8c).

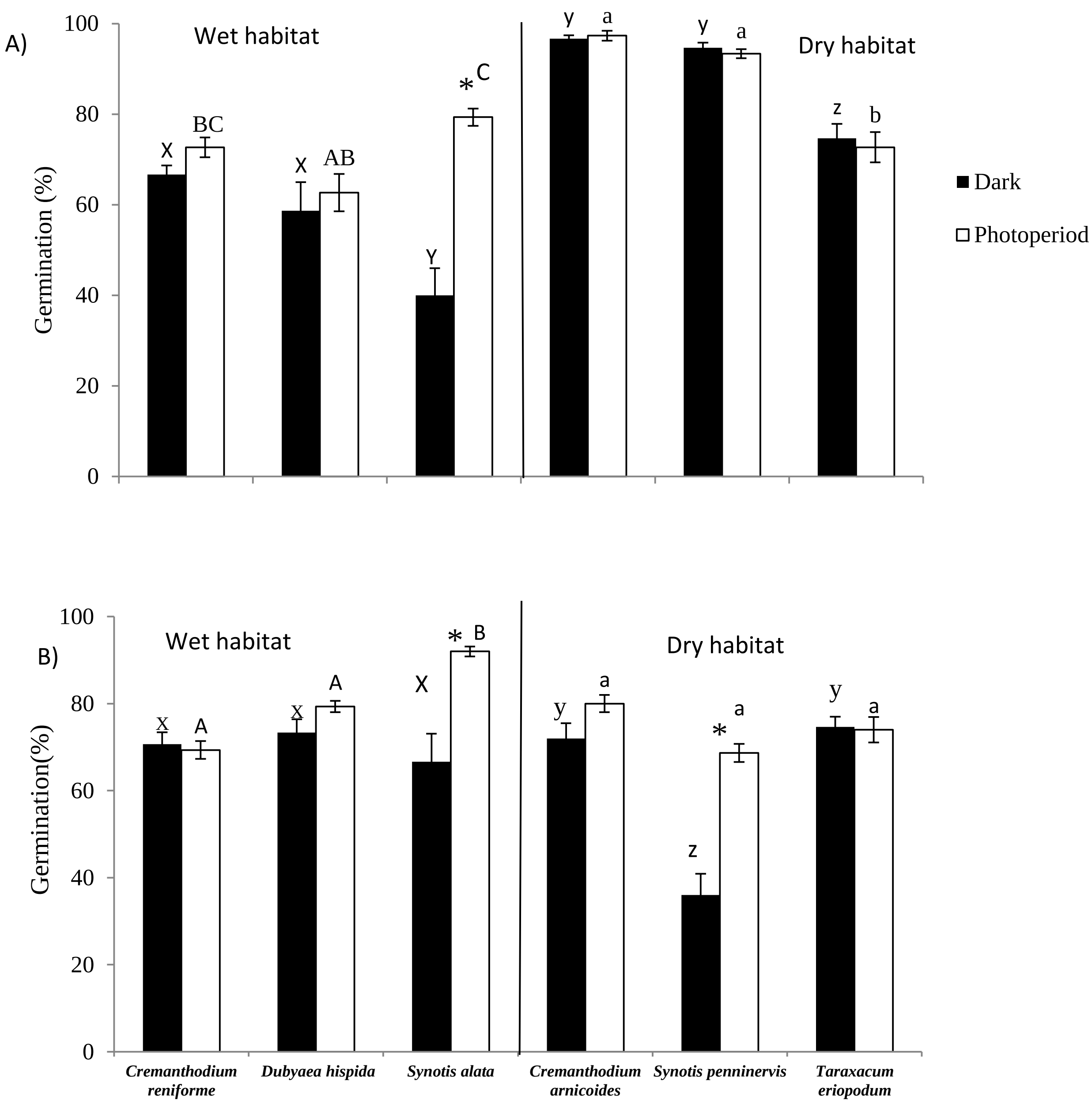

Figure 7. A) Germination percentage (GP) at 12h photoperiod and dark under low temperature (25/15°C) B) Germination percentage (GP) at 12h photoperiod and dark under high temperatures (30/20°C). n=5. Error bars represent ± SD of the mean. '*' represent significant difference at *p* ≤ 0.05 (t-test). '*' represent significant difference between 12h Photoperiod and dark within species. Capital and small letter represents significant difference between 12h photoperiod and dark among species.

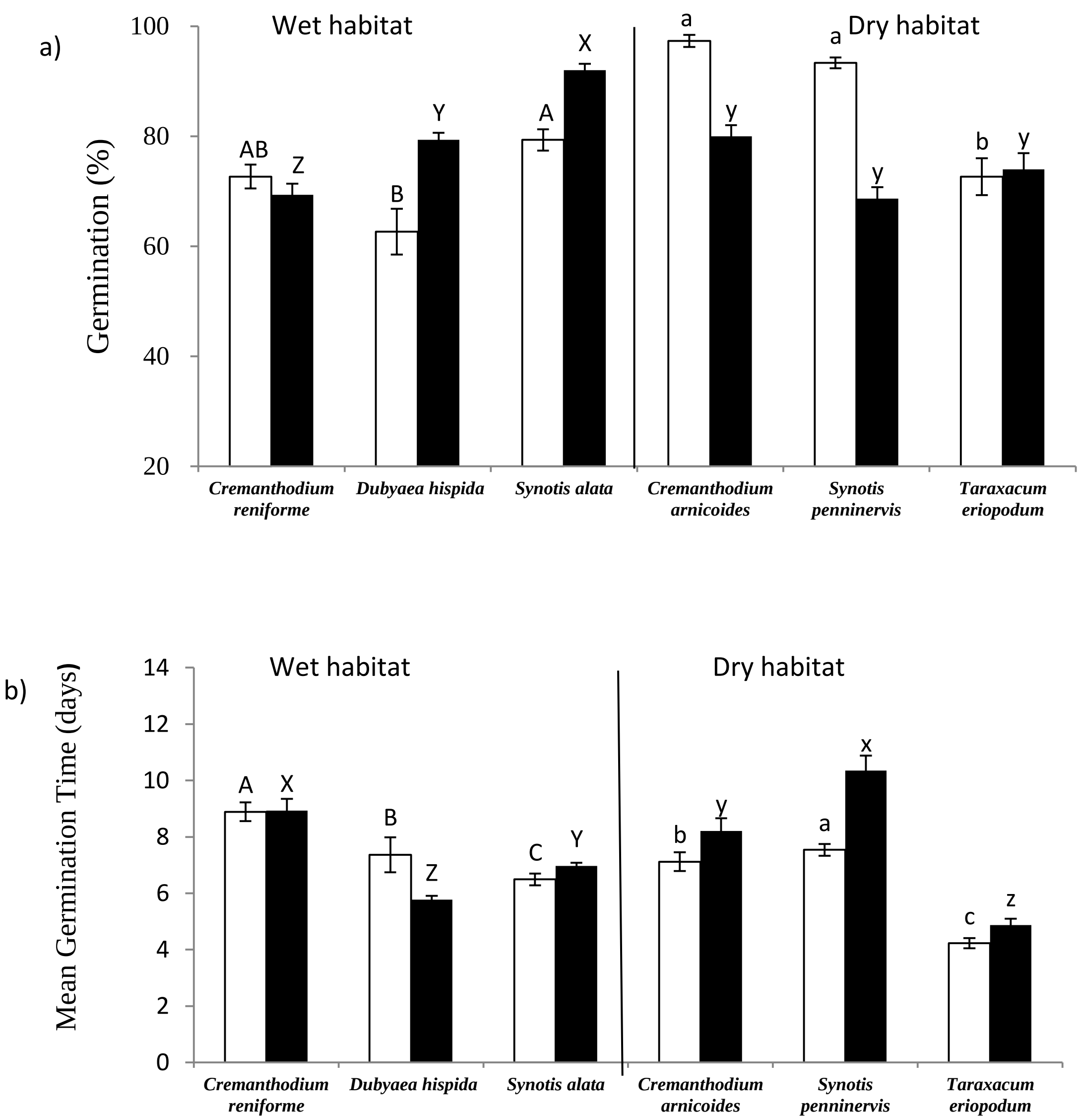

Figure 8. Effect of temperature on (a) Germination Percent (b) Mean Germination Time (MGT) in high (30/20°C) and low(25/15°C) temperatures, and error bars represent ±1 SE of the mean. Different alphabet A-c above the bar represent significant difference ($p \leq 0.05$) among species at low temperature; different alphabet X-z represent a significant difference ($p \leq 0.05$) among species at high temperature

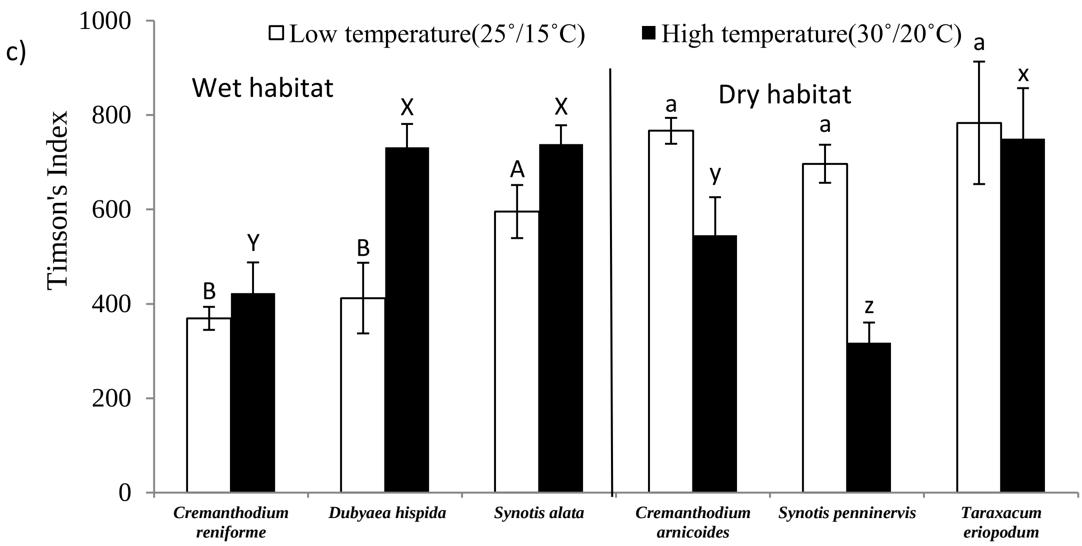

Figure 8. Effect of temperature on (c) Timson's Index (TI) in high (30/20°C) and low(25/15°C) temperatures, and error bars represent ±1 SE of the mean. Different alphabet A-c above the bar represent significant difference ($p \leq 0.05$) among species at low temperature; different alphabet X-z represent a significant difference ($p \leq 0.05$) among species at high temperature

4.2.3 Effect of water stress

The Germination Percentage (%) showed variation within all the tested species (from wet and dry regions) at different Water Potential values (0 MPa, -0.1 MPa and -0.25 MPa) (Fig. 9). At -0.5MPa water potential germination was significantly decreases. Germination of all six species was significantly higher in water potential at -0.25MPa. Germination percentage of *Synotis alata* and *Taraxacum eriopodum* remained similar at water potential 0, -0.1, -0.25 and -0.5MPa. Only seeds of *Cremanthodium arnicoides* were able to germinate at -0.75MPa and no germination was observed in water potential of -1MPa.

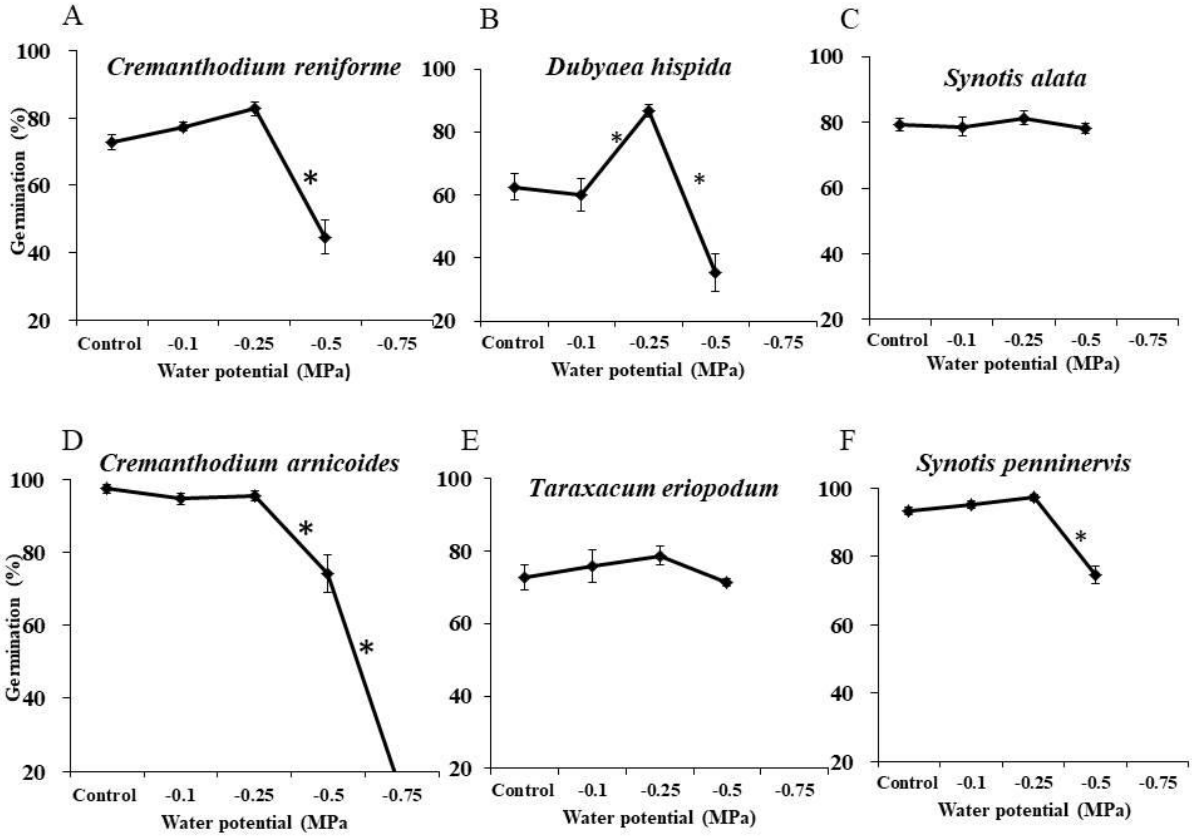

Figure 9. Effect of water stress on Germination percentage (GP) under low temperature (25/15°C) at photoperiod Error bars represent ± SD of the mean. n=5. '*' represent significance level at $p \leq 0.05$ (t-test). A-C represents wet region and D-F represents dry region.

Water stress negatively affected seed germination of species of high Himalaya at high temperature. Variation in the Germination Percentage (%) within the same species. i.e. *Synotis alata* and *Synotis penninervis* was also noted in the experiment trials involving different Water Potential values at a constant state of high temperature (30/20˚C). The Germination Percentage (%) of *Synotis penninervis* (68.67%) was found to be lower than *Synotis alata* (92% (Fig. 10). Germination percentage was significantly higher at water potential of -0.25MPa. After -0.25MPa germination was significantly lower at -0.5MPa water potential. Germination was not obtained at -0.75 and -1MPa water potential.

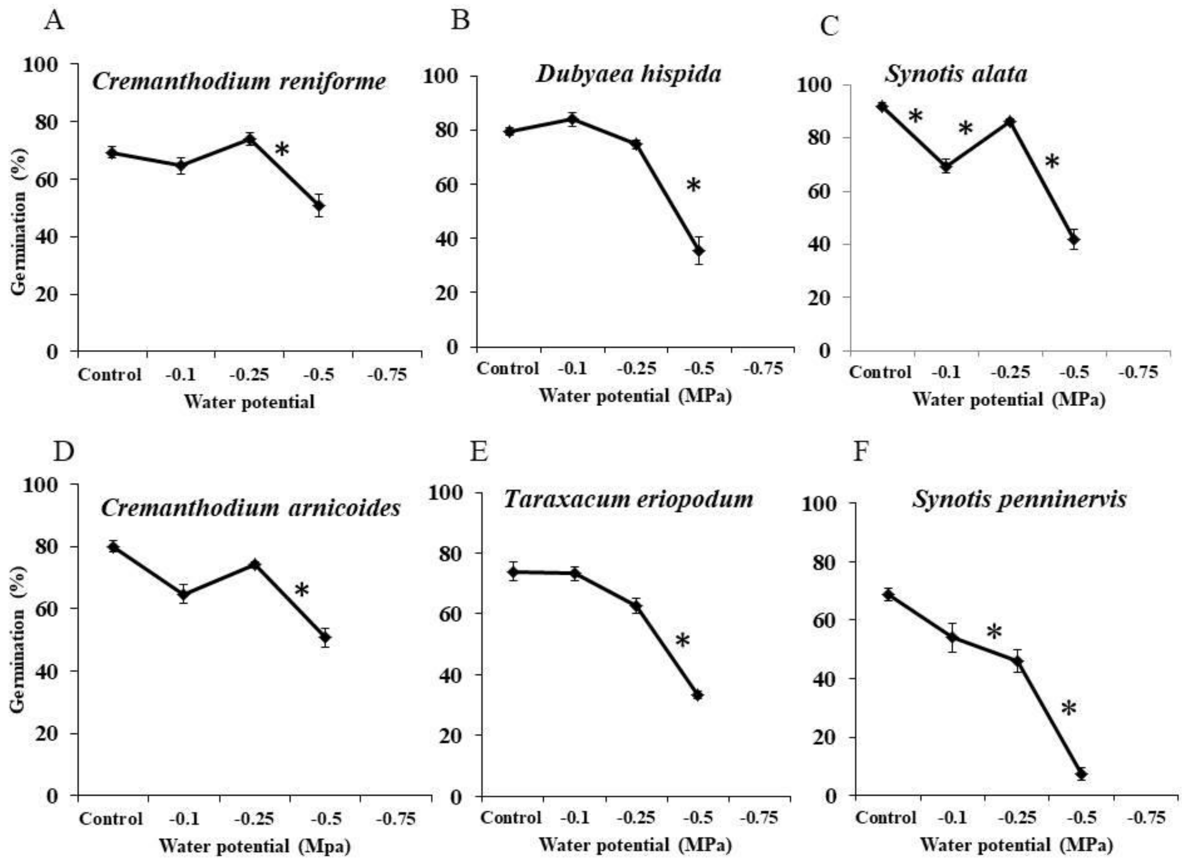

Figure 10. Effect of water stress on Germination percentage (GP) under high temperatures (30/20°C). Error bars represent ± SD of the mean. n=5. '*' represent significance level at $p \leq 0.05$ (t-test). A-C represents wet region and D-F represents dry region.

At low temperature (25/15˚C), Mean Germination Time (MGT) was higher in *Cremanthodium reniforme* which was followed by *Synotis penninervis* and lower was found in *Taraxacum eriopodum* (Fig.11). Out of six species only *Cremanthodium arnicoides* show significant difference at -0.75MPa.

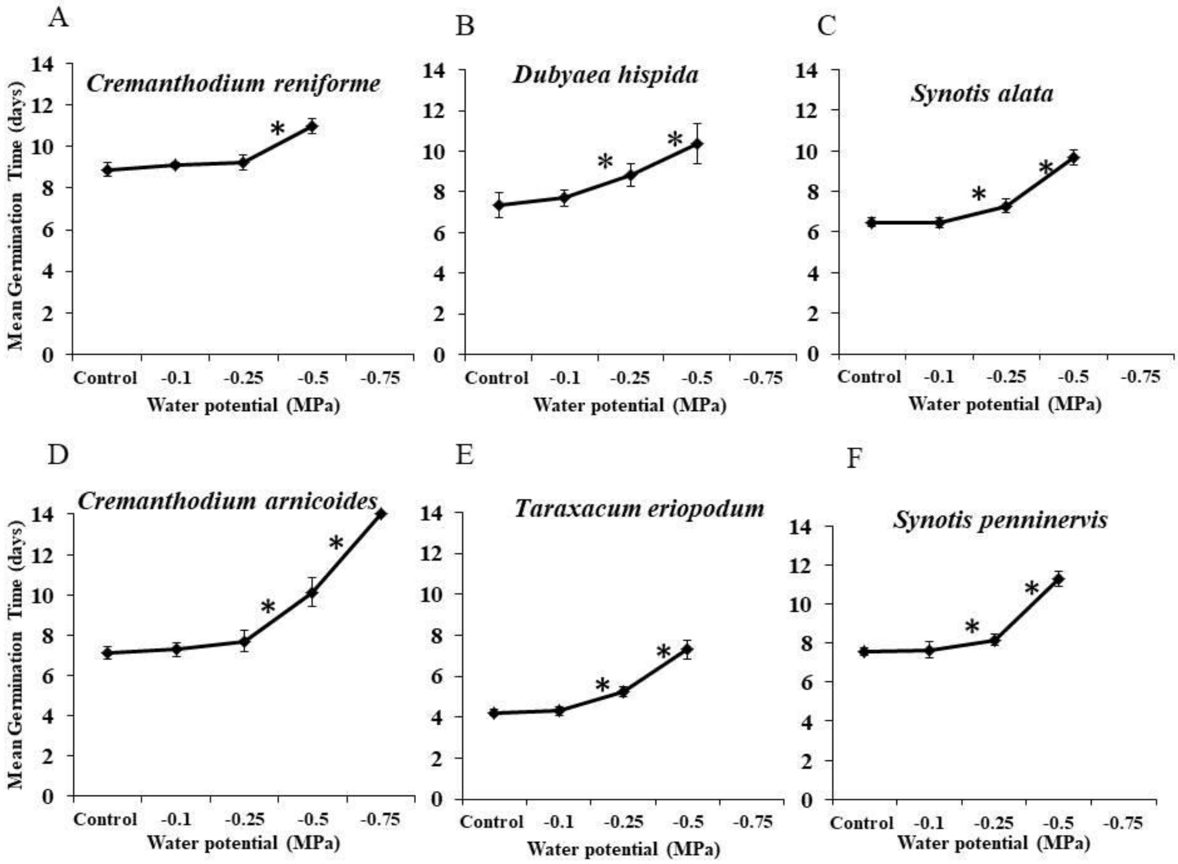

Figure 11. Effect of water stress on A) Mean germination time (MGT) under low temperature (25/15˚C). Error bars represent ± SD of the mean. n=5.'*' represent significance level at $p \leq 0.05$ (t-test). A-C represents wet region and D-F represents dry region.

At high temperature (30/20˚C), MGT was significantly higher in *Synotis penninervis* (Fig.12) which was followed by *Cremanthodium reniforme* and the least was found in *Taraxacum eriopodum*.

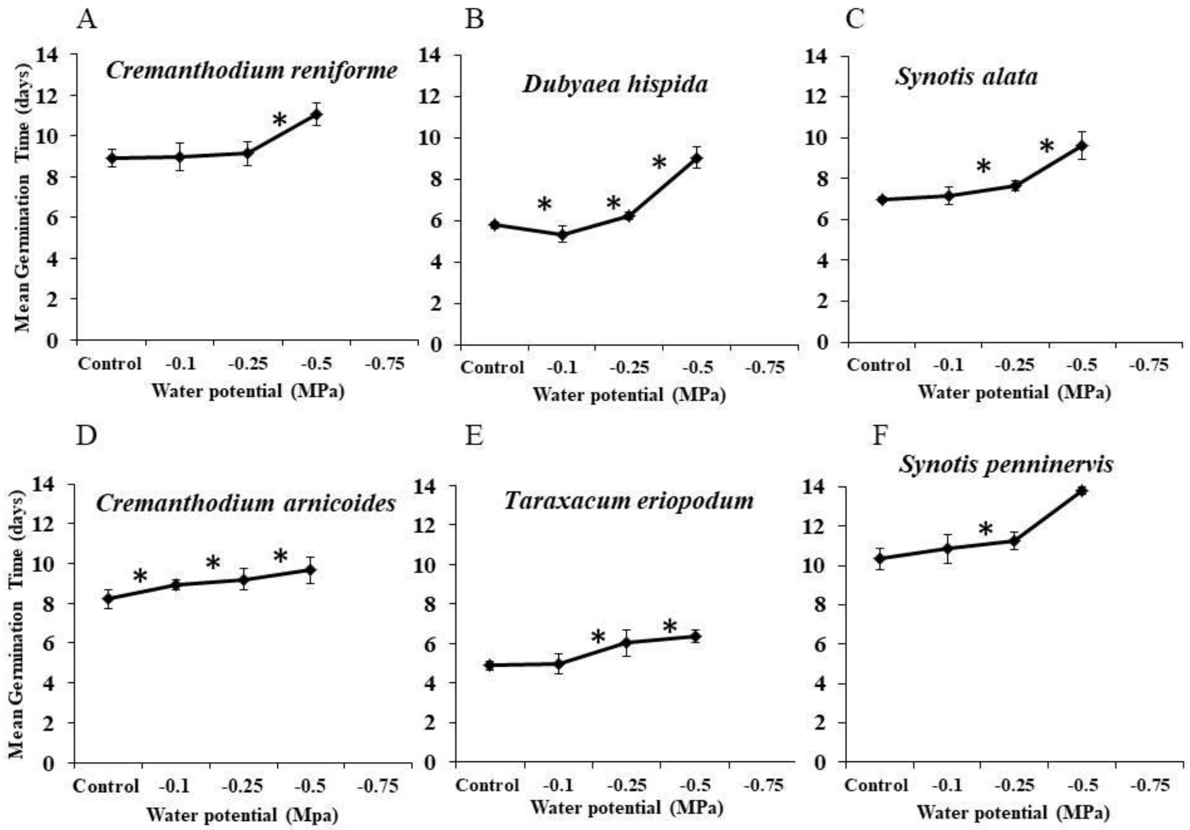

Figure 12. Effect of water stress on Mean germination time (MGT) under high temperature (30/20˚C). Error bars represent ± SD of the mean. n=5.'*' represent significance level at $p \leq 0.05$ (t-test). A-C represents wet region and D-F represents dry region.

At low temperature (25/15˚C), the significantly higher Timson's Index (TI) 800 was found in *Taraxacum eriopodum* which was followed by *Cremanthodium arnicoides* and *Synotis penninervis* (Fig.13). Significantly lower TI was found in *Dubyaea hispida* and *Cremanthodium reniforme*. Only *Cremanthodium arnicoides* show significant difference in TI at -0.75MPa.

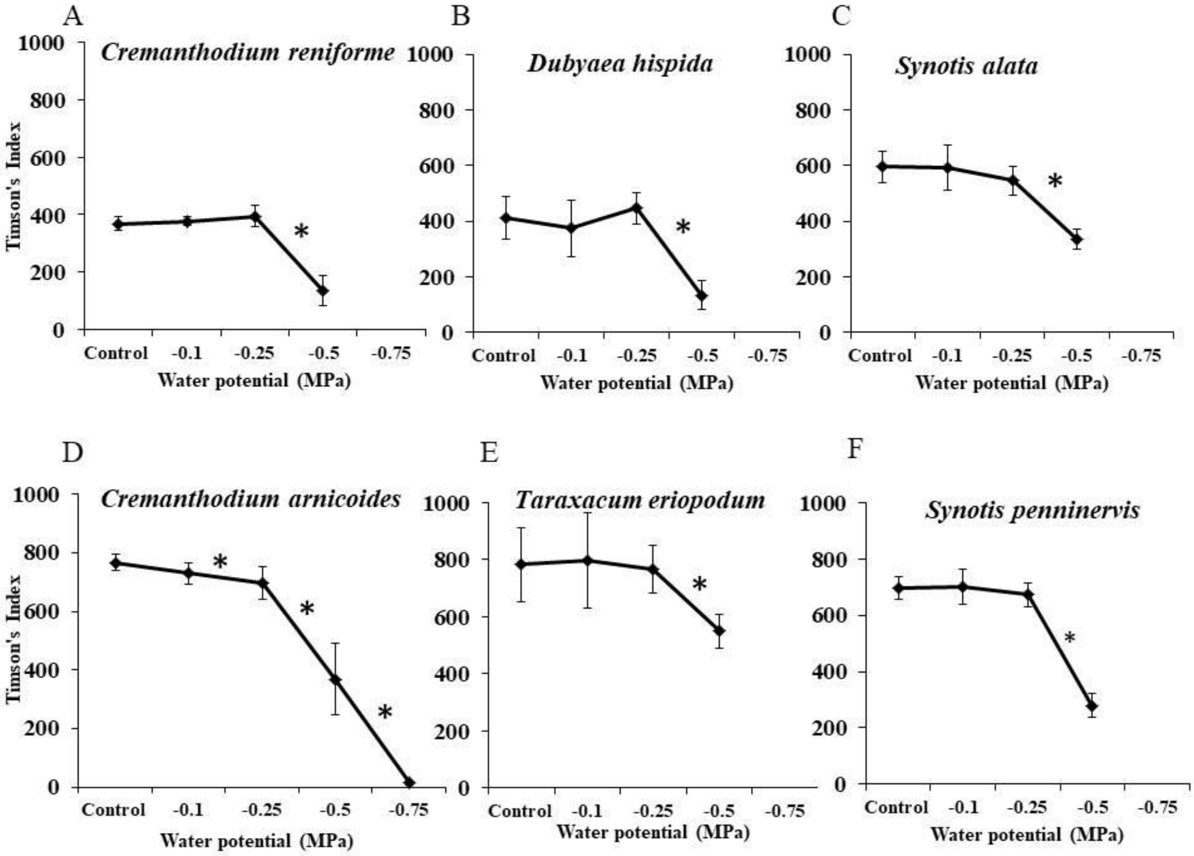

Figure 13. Effect of water stress on Timson's index (TI) under low temperature (25/15˚C). Error bars represent ± SD of the mean. n=5.'*' represent significance level at $p \leq 0.05$ (t-test). A-C represents wet region and D-F represents dry region.

At high temperature (30/20˚C), Timson's Index differed significantly among species. *Dubyaea hispida* showed significantly highest TI which was followed by *Taraxacum eriopodum* and the least was found in *Synotis penninervis* (Fig.14). Significant difference in TI was found in water potential at -0.25 and -0.5MPa.

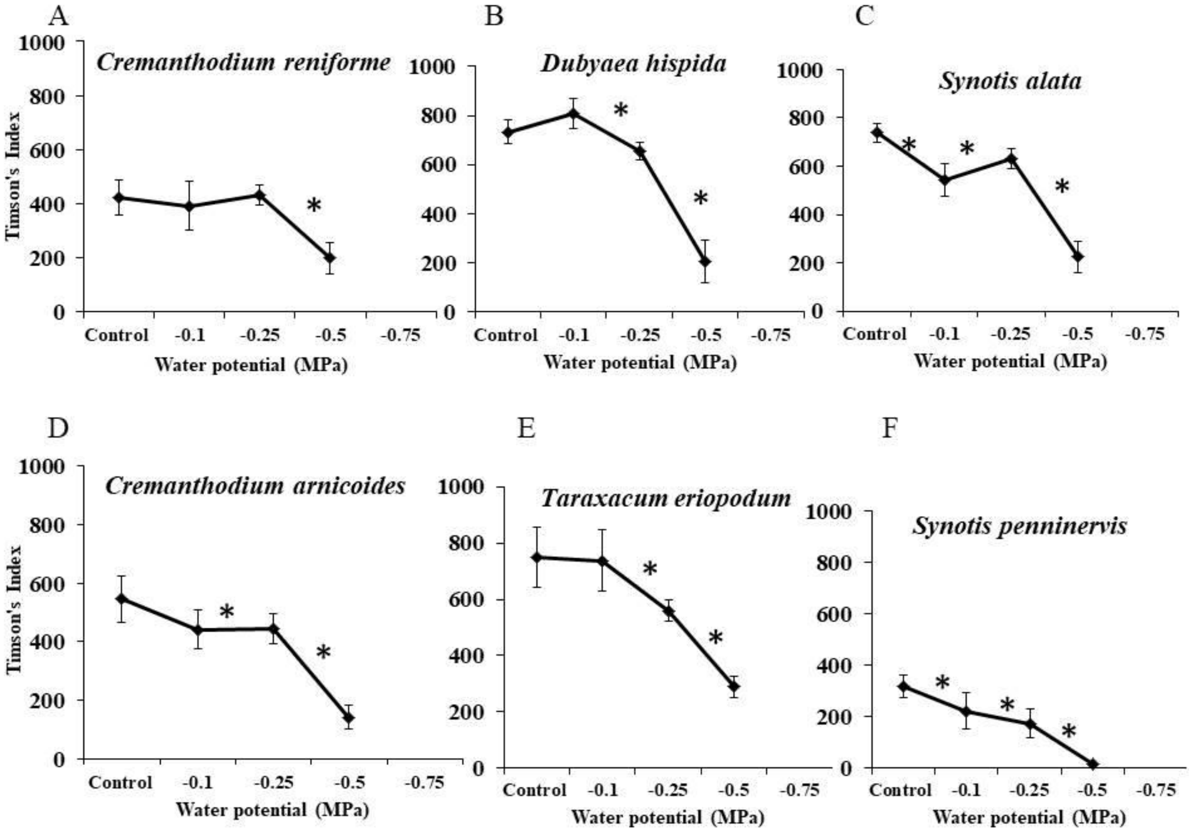

Figure 14. Effect of water stress on Timson's index (TI) under high temperature (30/20˚C). Error bars represent ± SD of the mean. n=5. '*' represent significance level at $p \leq 0.05$ (t-test). A-C represents wet region and D-F represents dry region.

At low temperature (25/15˚C), Germination percentage, Mean Germination Time and Timson's Index varied significantly with species, water potential as well as their interaction (Table 4A). Effect of species and water potential alone was significantly higher compared to their combined effect in all germination percentage, MGT and TI.

Similarly, at high temperature (30/20˚C) effect of species and water potential alone was also significantly higher (Table 4B) compared to their combined effect in all germination percentage, MGT and TI.

Table 4: Results of two-way ANOVA showing the effect of the interactions of species and water potentials on Germination percentage (GP), Mean germination time (MGT) and Timson's index (TI)

(A) Low temperature (25/15˚C)

	Variables	df	F-value	Sig.
Germination Percentage	Species	5	32.23	.000
	Water potential	3	30.68	.000
	Species×water potential	15	2.90	.001
Mean Germination Time	Species	5	236.94	.000
	Water potential	3	320.38	.000
	Species×water potential	15	3.0	.001
Timson's Index	Species	5	103.11	.000
	Water potential	3	128	.000
	Species×water potential	15	1.77	.050

(B) High temperature (30/20˚C)

	Variables	df	F-value	Sig.
Germination Percentage	Species	3	24.32	.000
	Water potential	5	128.43	.000
	Species×water potential	15	4.32	.000
Mean Germination Time	Species	3	24.98	.000
	Water potential	5	4.42	.006
	Species×water potential	15	1.8	.045
Timson's Index	Species	3	131.23	.000
	Water potential	5	245.93	.000
	Species×water potential	15	7.7	.000

CHAPTER 5: DISCUSSION

5.1 Seed Mass and Seed Size

Generally, a seed's access to resources is severely constrained in low-light or desert climatic environments. Growth in seed size has typically been linked to an increase in water availability (Castro, 1999). Small seeded species inhabit dry region where the water availability varies frequently. Also, these species ingest water quickly (Kikuzawa and Koyama, 1999). Additionally, species with smaller seeds tend to flourish in open or mesic environments than in low-light or arid habitats (Foster and Janson,1985; Mazer, 1989). Although there may be a positive (Sultan and Bazzaz, 1993) or negative (Westgate *et al*., 1989) association, seed size has frequently been linked to the severity of drought stress. In this study smaller seed size was found in *Taraxacum eriopodum* because of the moisture content in dry region. Larger seed size was found in *Dubyaea hispida* from moist region which can be attributed to abundant water availability (Kikuzawa and Koyama, 1999).

In wet region seed mass was higher because the growing season was long (Rockwood, 1985). Opposite of this trend was noted in this study because higher seed mass was found in *Cremanthodium arnicoides* from dry region because the need for successful establishment produces selection pressure for large seeds (Kidson & Westoby, 2000). Lower seed mass was found in *Taraxacum eriopodum* from dry region because small-seed evolution might benefit from widespread distribution (Fenner, 1983). The availability of moisture and seed mass may not always be correlated (Telenius and Torstensson, 1991).

Seed size differs enormously among plant species with mean masses differing from< 10^{-6} to 10^{4}g (Harper, 1977). The availability of resources (i.e., soil moisture levels) is likely to have an impact on seed size and number (Parciak, 2002). There is trade-off between small seeded species vs. large seeded species.

5.2 Effect of temperature and light

Light and temperature markedly influence the seed germination patterns. Temperature may be a single environmental component that has the greatest impact on their germination (Bailly, 2019). Species on sunny, arid slopes of Manang, dry region did not significantly respond to changing temperatures in terms of germination (Liu *et al*., 2013). In present study, the higher germination percentage was of *Cremanthodium arnicoides* and *Synotis penninervis* from dry region and the lowest was found in *Dubyaea hispida* from moist region at low temperature (25/15℃). Seeds of plants that prefer dry environments are likely capable of sensing the level of moisture in their surroundings, preventing germination in adverse field circumstances (Ludewig *et al*., 2014). So, plants growing on dry, sunny slopes were less likely to respond favorably to temperature changes (Liu *et al*., 2013). Generally, warm winter specimens sprouted quickly compared to delayed germination of cold winter collections (Meyer and Monsen, 1991).

In this research, the result of *Cremanthodium arnicoides* and *Synotis penninervis* is similar Liu *et al* (2013). At low temperature (25/15℃) seeds had higher germination percentage as compared to high temperature because low temperature delays the embryonic activities whereas high temperature destroys the delicate embryo tissue (Macova *et al*., 2022). In the last phases of seed maturation, high temperatures and increasing moisture stress both reduce the levels of dormancy in wild oat seeds (Naylor, 1983). Temperature influences germination by adjusting dormancy and rapid of germination in non-dormant seeds (Baskin and Baskin, 2014). Effect of temperature in germination of seeds differs among plant species. *Senecio coincyi* germinated 100% at temperature 25℃ (Martinez-Garcia *et al*., 2012). Two weeds such as *Silybum marianum* and *Avena fatua* had higher germination percentage at 25℃. Seeds of *Brachycome muelleri* couldn't germinate at >20℃ temperature (Jusaitis *et al*., 2004). Seeds of *Chromolaena odorata* had higher germination percentage at optimum temperature 25℃ (Ping *et al*., 2011). Ornamental sunflowers germinated >87% under 15-30℃ temperature (Toscano *et al*., 2017). According to Hu *et al*., 2015, the seed germination of *Ammopiptanthus mongolicus*, *Glycyrrhiza uralensis*, *Lespedeza potaninii*, and *Sophora alopecuroides* from the regions of higher and warmer temperatures with low rainfall region, and *Vicia amoena*, *Vicia angustifolia*, *Vicia sativa* and *Vicia unijuga* from low temperatures regions, having proportionally ample rainfall surroundings suggests that the base mean water potential vary between species but not among regions.

In present study seeds from the moist region germinated better at high temperature. At high temperature (30/20℃) higher germination percentage was found in *Synotis alata* and the lowest was in *Synotis penninervis*. Compared to seeds from warm, dry regions, seeds from *Stipa* species grew better in cool, damp environments (Zhang *et al*., 2020). At greater temperature there is decrease in germination rate (Clavijo and Medina, 2004). High endogenous abscisic acid contents was related with high temperature that inhibit germination (Toyomasu *et al*., 1993).

At a well define range, germination increased with increase in temperature and then germination decreased with further increase in temperature (Alvarado and Bradford, 2002). Such trend was noticed in the present research. At 25/15℃ germination rate was found to be higher but it decreased in further increasing temperature in *Cremanthodium reniforme*, *Cremanthodium arnicoides* and *Synotis penninervis*. Other species *Dubyaea hispida*, *Synotis alata* and *Taraxacum eriopodum* germinated greater at high temperature (30/20℃). Heimann and Cussans (1996) also reported that *Circium arvensis* germinated greater at high temperature (25-30℃).

Seeds of many Asteraceae species required light for germination (Galindez *et al*., 2009). Under 12 h photoperiod conditions seeds were germinated at both low and high temperature. All seeds were non photoblastic. At low temperature (25/15℃) *Cremanthodium arnicoides* and *Synotis penninervis* had similar germination percentage in both photoperiod and dark conditions. Open habitat provides favourable light and warmth for the advanced germination of seeds (Brink *et al*., 2012). This trend was also found in the present study because seeds from dry habitat germinated well in light condition. Large seeded species have greater germination percentage, emergence or establishment than small seeded species (Dalling *et al*., 1998). In this research, large seeded species *Cremanthodium arnicoides* obtained greater germination percentage in comparison to small seeded species *Taraxacum eriopodum*. This showed that small seeded species require more light to germinate in comparison to large seeded species (Milberg *et al*., 2000). Seed size and seed structure independently influence germination (Zhang, 1993).

Larger seeds have ample food reserves, allowing quick emergence and higher initial seedling size with greater tolerance to shadow, drought and physical destruction (Leishman *et al*., 2000). Likewise, smaller seeds can also germinate quickly than larger seeds to obtain competitive advantage (Grime *et al*., 1981). Larger seeds require light to germinate in the existence of changing temperature (Thompson and Grime, 1983). Several previous studies have also examined the role of light in germination. For example, seeds of *Echinacea purpurea* obtained greater germination percentage when prechilled in light in compared to dark (Li *et al*., 2007). Seeds of *Artemisia* species had higher germination percentage in light as compared to dark (Li *et al*., 2012). *Ipomeoa triloba* a weed species can germinate well in both light and dark (Chauhan and Abugho, 2012) but germination of *Leptochloa chinensis* was fully suppressed in the dark (Chauhan and Johnson, 2008). Light is not needed for germination of *Echinochloa crusgalli* and *Echinochloa colona*, but it provokes their germination (Chauhan and Johnson, 2009, 2011). In present study, selected plant species had high and nearly equal germination percentage in both dark and photoperiod. According to Baskin and Baskin (1998), many species can germinate equally well in both light and darkness, however certain species can germinate to larger percentages in darkness than in light (Koller, 1957; Keren and Evenari, 1974).

At high temperature (30/20℃), *Synotis alata* and *Synotis penninervis* germinated well in light as compared to dark. It meant that those seeds needed light for better germination. Similarly other species such as *Cremanthodium reniforme*, *Dubyaea hispida*, *Cremanthodium arnicoides* and *Taraxacum eriopodum* germinated nearly equal in both photoperiod and dark conditions. It meant that seeds can germinate well in both light and dark condition (Baskin and Baskin, 1998).

5.3 Effect of water stress

At low temperature (25/15℃), under high water stress condition (≤-0.5 MPa), *Cremanthodium reniforme* and *Dubyaea hispida* had lower germination percentage than other four species. This might be because these two species grows naturally in very moist area. The germination of plant species representing wet regions may be reduced at lower water potentials more than that of plant species from dry regions (Ludewig *et al*., 2014). This trend was found in the present research because species from moist habitat had lower germination percentage than dry habitat. Seeds of plants that prefer dry environments are likely capable of sensing the level of moisture in their surroundings, preventing germination in adverse field circumstances (Ludewig *et al*., 2014). Dry habitat may have more moisture-sensing mechanisms (Ludewig *et al*., 2014). We hypothesized that seeds of species from the dry region (Manang) should have more stress tolerant than those from the wet region (Annapurna Base Camp) and the hypothesis was accepted because, *Cremanthodium arnicoides* germinated up to -0.75MPa water potential, which were collected from dry region indicated that its capacity to tolerant water stress was higher than other five species.

Toscano *et al*., (2017) reported that hybrid sunflower "Zohar" seeds also germinated at -0.75MPa. Mexican sunflower seeds which tolerate high water stress germinated higher in high temperature and water stress (Wen *et al*., 2015). Species adapted to harsh surroundings can be less influenced by water stress than these adapted to moist environments (Allen *et al*., 2000). There was no response to osmotic potential in relation to habitat types (Daws *et al*., 2008). In different plant species, water stress may delay or reduce seed germination (Zhou *et al*., 2005). In present study, germination rate was higher at -0.25MPa as compare to other water potential. Seed germination reaction to water potential are species specific (Fenner and Thompson, 2005; Baskin and Baskin, 2014). Effect of water deficiency caused delaying and decrease of seed germination (Alvarado and Bradford, 2002). In present study, there was higher germination percentage of Himalayan wild species at -0.25MPa concentration. Furthermore, comparing between the congeners, *Cremanthodium arnicoides* germinated better than the *Cremanthodium reniforme*; similarly *Synotis penninervis* performed better than the *Synotis alata* at -0.25 MPa. There was no germination of *Cremanthodium reniforme* at -0.75Mpa. This indicated that *Cremanthodium arnioides* and *Synotis penninervis* could be a better stress tolerant species than its congener species.

When compared to species that are acclimated to wet habitats, species that are adapted to dry conditions may be less sensitive to water stress during germination (Allen *et al*., 2000). Low water potentials did not encourage the germination of many wetland species, but they did encourage the germination of several dry environment species (Evans and Etherington, 1990). At arid environments, seed germination can be less influenced by water stress than those adjusted to moist environments (Allen *et al*., 2000). Seed germination of plant species from the wet habitats shows a positive relationship with the seed size as large-seeded species are generally more adaptive to a wide range of environmental conditions (Manasse, 1990).

At high temperature (30/20°C), germination was also better in -0.25MPa as compared to other water potential. *Synotis alata* had higher germination percentage whereas *Synotis penninervis* had lower germination percentage at -0.25MPa. No germination was occured at -0.75 MPa. At high temperature and high water stress *Synotis penninervis* could not germinated well in this study, all species could be tolerant as a moderate level of water stress.

Overall, results of this study suggest that at low temperature *Cremanthodium arnicoides* and *Synotis penninervis* germinated better as compared to other four species. This was evident from a higher germination percentage, higher mean germination time under various environmental conditions such as temperature, moisture, light, radiation and water stress. The results also suggest germination pattern of species may be modified by changing environment at local and regional levels.

CHAPTER 6. CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Among the all six species *Cremanthodium arnioides*, *Synotis penninervis* and *Taraxacum eriopodum* from dry region could be a better stress tolerant species than its congener species. Seed mass was higher in species from dry region than from moist region. Germination was well in both photoperiod and continuous dark conditions. Under different environmental conditions such as photoperiod/continuous dark and high and low temperature, seed germination percentage of species from dry region was greater in low temperature and photoperiod. But in case of water stress only *Cremanthodium arnicoides* seeds germinated at -0.75MPa and none of the test species there was germinated at -1MPa. The differences in germination among closely related congeneric species reflect seed germination pattern of species found in regions with contrasting environment. Information from germination experiment may serve as the base line for prediction of future distribution and survival of high Himalayan Asteraceae species. Overall the capacity to germinate under wide range of light, temperature and water stress contributed to species adjustment, tolerant and distribution of species. In conclusion the species from dry region could tolerate higher stress in different temperature compared to moist region that meant the set hypothesis of this study is accepted which is species from dry region have higher stress tolerance than species from wet region. The result also suggest that germination pattern of species may be modified by changing environment at local and regional levels.

6.2 Recommendation

Seed germination and seed traits of Himalayan wild species in two different regions are unknown. Additional study will help to determine how Himalayan wild species will adapt to climate change and survive.

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APPENDICES

Appendix 1: Germination percentage at photoperiod and continuous dark at low temperature (25/15°C)

Species	Photoperiod	Dark
Cremanthodium reniforme	21.8±2.16^{BC}	20±2^{X}
Dubyaea hispida	18.8±4.15^{AB}	17.6±6.35^{X}
Synotis alata	23.8±1.92^{*C}	12±5.96^{*Y}
Cremanthodium arnicoides	29.2±1.09^{a}	29±0.71^{y}
Synotis penninervis	28±1^{a}	28.4±1.14^{y}
Taraxacum eriopodum	21.8±3.35^{b}	22.4±3.21^{z}

Note: * represents significant difference (p<0.05).

Appendix 2: Germination percentage at photoperiod and continuous dark at high temperature (30/20°C)

Species	Photoperiod	Dark
Cremanthodium reniforme	20.8±2.05^{A}	21.2±2.77^{X}
Dubyaea hispida	23.8±1.30^{A}	22±3.08^{X}
Synotis alata	27.6±1.14^{*B}	20±6.40^{*X}
Cremanthodium arnicoides	24±2^{a}	21.6±3.51^{y}
Synotis penninervis	20.6±2.07^{*a}	10.8±4.92^{*z}
Taraxacum eriopodum	22.2±2.94^{a}	22.4±2.30^{y}

Note: * represents significant difference (p<0.05)

Appendix 3: Germination percentage at low temperature (25/15°C) and at high temperature (30/20°C)

Species	Low temperature	High temperature
Cremanthodium reniforme	21.8 ± 2.16^{AB}	20.8 ± 2.05^{Z}
Dubyaea hispida	18.8 ± 4.15^{B}	23.8 ± 1.30^{Y}
Synotis alata	23.8 ± 1.92^{A}	27.6 ± 1.14^{X}
Cremanthodium arnicoides	29.2 ± 1.09^{a}	24 ± 2^{y}
Synotis penninervis	28 ± 1^{a}	20.6 ± 2.07^{y}
Taraxacum eriopodum	21.8 ± 3.35^{b}	22.2 ± 2.94^{y}

Note: Alphabet represents significant difference ($p<0.05$).

Appendix 4: Mean germination time at low temperature (25/15°C) and at high temperature (30/20°C)

Species	Low temperature	High temperature
Cremanthodium reniforme	8.89 ± 0.34^{A}	8.93 ± 0.41^{X}
Dubyaea hispida	7.36 ± 0.62^{B}	5.77 ± 0.14^{Z}
Synotis alata	6.49 ± 0.21^{C}	6.97 ± 0.11^{Y}
Cremanthodium arnicoides	7.12 ± 0.33^{b}	8.21 ± 0.45^{y}
Synotis penninervis	7.54 ± 0.21^{a}	10.36 ± 0.53^{x}
Taraxacum eriopodum	4.23 ± 0.18^{c}	4.87 ± 0.22^{z}

Note: Alphabet represents significant difference ($p<0.05$).

Appendix 5: Timson's Index at low temperature (25/15°C) and at high temperature (30/20°C)

Species	Low temperature	High temperature
Cremanthodium reniforme	369.3 ± 24.3^{B}	422.6 ± 65.4^{Y}
Dubyaea hispida	412 ± 74.9^{B}	732 ± 48.8^{X}
Synotis alata	595.3 ± 56.1^{A}	738.6 ± 39.9^{X}
Cremanthodium arnicoides	766.6 ± 27.3^{a}	545.3 ± 80.3^{y}
Synotis penninervis	696.7 ± 40.5^{a}	318 ± 42.4^{z}
Taraxacum eriopodum	783.3 ± 129.8^{a}	750 ± 106.7^{x}

Note: Alphabet represents significant difference ($p<0.05$).

Appendix 6: Germination percent (GP), Mean Germination Time (MGT), Timson's Index (TI) (Mean ± S.E.) at different water potentials at low temperature (25/15°C)

Species		Water potential				
		0	-0.1	-0.25	-0.5	-0.75
Cremanthodum reniforme	GP	21.8±2.16	23.2±1.30	24.8±1.92	13.4±4.92*	0
	MGT	8.89±0.33	9.12±0.14	9.23±0.36	10.94±0.36*	0
	TI	369.33±24	378±17.09	393.99±37	137.33±52.6*	0
Dubyaea hispida	GP	18.8±4.15	18±5.15	26±2*	10.6±5.89*	0
	MGT	7.36±0.62	7.70±0.39	8.83±0.53*	10.36±1*	0
	TI	412±75	374.66±101	447.33±56.78	116±51.98*	0
Synotis alata	GP	23.8±1.9	23.6±2.96	24.4±2.19	23.4±1.52	0
	MGT	6.49±0.2	6.46±0.23	7.28±0.35*	9.68±0.4*	0
	TI	595.3±56	593.34±81	547±51.23	336.6±37*	0
Cremanthodium arnicoides	GP	29.2±1.1	28.4±1.51	28.6±1.34	22.2±5.2*	4.2±1.6*
	MGT	7.12±0.3	7.23±0.34	7.69±0.52	10.1±0.8*	14±0*
	TI	766.6±27	729.3±36.3	696±54.95*	368±123*	14±5.5*
Synotis penninervis	GP	28±1	28.6±1.14	29.2±0.84	22.4±2.6*	0
	MGT	7.54±0.2	7.64±0.41	8.07±0.30*	11.3±0.4*	0
	TI	696.6±40	702.6±61.7	674±41.86	279.3±42*	0
Taraxacum eriopodum	GP	21.8±3.4	22.4±4.56	23.6±2.51	21.4±1.14	0
	MGT	4.23±0.2	4.31±0.22	5.24±0.25*	7.31±0.5*	0
	TI	783±123	799±167.28	768±84.35	549±59.6*	0

Note: * represents significant difference ($p<0.05$).

Appendix 7: Germination percent (GP), Mean Germination Time (MGT), Timson's Index (TI) (Mean ± S.E.) at different water potentials at high temperature (30/20°C)

Species		Water potential				
		0	-0.1	-0.25	-0.5	-0.75
Cremanthodium reniforme	GP	20.8±2.05	19.4±2.88	22.2±2.05	15.2±3.96*	0
	MGT	8.92±0.41	8.96±0.66	9.14±0.57	11.08±0.57*	0
	TI	422.6±65.4	392.66±90	431.33±36	199.33±57*	0
Dubyaea hispida	GP	23.8±1.30	25.2±2.58	22.4±1.52	10.6±5.02*	0
	MGT	5.77±0.13	5.34±0.37*	6.22±0.18*	9.03±0.52*	0
	TI	732±48.85	809.33±61	655.33±36*	206±86*	
Synotis alata	GP	27.6±1.14	20.8±2.48*	25.8±1.09*	12.6±3.84*	0
	MGT	6.97±0.11	7.16±0.44	7.65±0.23*	9.59±0.67*	0
	TI	738.66±40	542.66±70*	632±42.79*	224±64.22*	0
Cremanthodium arnicoides	GP	24±2	21.8±3.03	23±1	8.2±2.94*	0
	MGT	8.2±0.45	8.92±0.25*	9.2±0.51*	9.67±0.67*	0
	TI	545.34±80	442±67.52	445±52.41*	142±42*	0
Synotis penninervis	GP	20.6±2.07	16.2±5.11	13.8±3.89*	2.2±2.04*	0
	MGT	10.35±0.53	10.85±0.73	11.24±0.45*	13.8±0.17	0
	TI	318±42.39	222.66±71*	172.66±56*	14.44±1.92*	0
Taraxacum eriopodum	GP	22.2±2.94	22±2.23	18.8±2.48	10±1.22*	0
	MGT	4.87±0.22	4.98±0.51	6.02±0.66*	6.36±0.31*	0
	TI	750±107	737.33±110	565.33±37*	288±39.55*	0

Note: * represents significant difference ($p<0.05$).

Germination of seeds under 12hrs photoperiod

Germination of seeds under complete dark

Germination of seeds under 12hrs photoperiod

Germination of seeds under complete dark

नेपाल सरकार
वन तथा वातावरण मन्त्रालय
राष्ट्रिय निकुञ्ज तथा वन्यजन्तु संरक्षण विभाग
(.................................... शाखा)
फोन नं. : ४२२०८५० ४२२०९१२ ४२२७९२६
फ्याक्स नं. ४२२७६७५
पो. ब. नं. – ८६०
बबरमहल, काठमाडौं
Email: info@dnpwc.gov.np
http//:www.dnpwc.gov.np

पत्र संख्या :- 32 ०७८।७९ इको
चलानी नं :- 280

मितिः २०७८/५/२०

विषयः अध्ययन अनुमति सम्बन्धमा ।

श्री अन्नपूर्ण संरक्षण क्षेत्र आयोजना, हरियोखर्क, पोखरा ।

प्रस्तुत विषयमा तहाँ संरक्षण क्षेत्रमा निम्नानुसारको अध्ययन अनुसन्धान अनुमति प्रदान गरिएको व्यहोरा निर्देशनानुसार अनुरोध छ ।

अनुसन्धानकर्ताको नाम	Bharat Babu Shrestha		
ठेगाना	शहिद लखन थापा गाउँपालिका-०९, गोर्खा	इमेलः Shresthabb@gmail.com	फोन नं. ९८४१२४१४८४
समद्व संस्था	वनस्पतिशास्त्र केन्द्रीय विभाग, त्रिभुवन विश्वविद्यालय, किर्तिपुर, काठमाण्डौ ।		
अनुसन्धानको प्रकृती	व्यक्तिगत		
पद	सह-प्रध्यापक		
अनुसन्धानको तह	राष्ट्रिय स्तर		
अनुसन्धानको शिर्षक	**Impact of climate change on germination, growth, pollination and distribution of Himalayan medicinal herbs *Aconitum spicatum* and *A. naviculare***		
अनुसन्धान विधि	Transplant Experiment in field	नमुना संकलन गर्ने	नमुना परिक्षण कहाँ गर्ने नेपालमा
अनुसन्धानको अविध	२० भाद्र २०७८ देखि १९ भाद्र २०८० (दुई बर्ष)		

शर्त :

१.अनुसन्धानकर्ताले राष्ट्रिय निकुञ्ज तथा वन्यजन्तु संरक्षण ऐन, २०२९ र नियमावली, २०३० तथा मातहतका सवै नियमावलीहरुको पूर्ण पालना गर्नु पर्नेछ ।

२. अध्ययन गर्दा सम्बन्धित संरक्षित क्षेत्र कार्यालयसंग समन्वय गरी कार्यालयमा कार्यरत कर्मचारीको रोहबरमा गर्नु पर्ने पर्नेछ ।

३. अनुसन्धानकर्ताले आफ्नो अनुसन्धानको प्रस्ताव सम्बन्धित संरक्षित क्षेत्र कार्यालयमा समेत पेश गर्नु पर्नेछ ।

४.अनुसन्धानकर्ताले अनुसन्धान समाप्त भएपछि प्राप्त तथ्याङ्क, एक प्रति कागजी प्रतिवेदन र एक प्रति इलोक्ट्रोनिक प्रतिवेदन यस विभाग र सम्बन्धित संरक्षित क्षेत्र कार्यालयमा बुझाउनु पर्नेछ ।

५. अनुसन्धानकर्ताले नतिजाहरु प्रकाशित गर्दा अनुसन्धानमा संलग्न यस विभाग र अन्तरगतका कर्मचारीको योगदानको आधारमा सहलेखकको रुपमा समावेश गराउनु पर्नेछ ।

६. सङ्कलित नमुना विदेश लैजान पाईने छैन ।

७. तोकिएका शर्तहरुको पालना नगरेमा विभागले कुनैपनि समयमा अनुमतिपत्र रद्द गर्न सक्नेछ ।

हेम राज आचार्य
सहायक इकोलोजिष्ट

बोधार्थः

श्री **Bharat Babu Shrestha:** सम्बन्धित संरक्षित क्षेत्र कार्यालयसंग समन्वय गरी अध्ययन अनुसन्धान गर्नु हुन र अनुसन्धान समाप्त भएपछि एक प्रति कागजी तथा विद्युतीय प्रतिवेदन सम्बन्धित कार्यालय र विभागमा वुझाउनु हुन अनुरोध छ ।

श्री अन्नपूर्ण संरक्षण क्षेत्र सम्पर्क अधिकारीको कार्यालयः जानकारीको लागि अनुरोध छ ।

वनस्पतिशास्त्र केन्द्रीय विभाग, त्रि.विः जानकारीको लागि अनुरोध छ ।

NATIONAL TRUST FOR NATURE CONSERVATION

ANNAPURNA CONSERVATION AREA PROJECT

Headquarters, Pokhara

Headquarters, Pokhara

Ref: 80 /078/079 Date: 2078-05-30

Dr. Bharat Babu Shrestha
Associate Professor
Central Department of Botany
TU, Kathmandu

Re: Permission to conduct research in Annapurna Conservation Area

We received your request letter regarding permission to conduct research on **"Impact of climate change on germination, growth, pollination, and distribution of Himalayan medicinal herbs *Aconitum spicatum and A. naviculare*"**. You have been given permission to carry out your field research in ACA with the following terms and conditions.

1. The research must be for scientific and academic purpose with the aim of making contribution in conservation and development of conservation area.
2. This permission will be **valid up to August 31, 2023** (2080 Bhadra 14).
3. You have to follow the ACAP Minimum Impact Code and the Conservation Area Management Regulation 2053.
4. You have to follow the terms and conditions mentioned in the research permit provided by Department of National Park and wildlife Conservation.
5. You are **allowed to collect sample only** from the study area.
6. You will have access to the NTNC-ACAP Resource Library in Pokhara.
7. Upon the completion of the research, **you must submit a hard copy and digital copy of your report** to the NTNC-ACAP Headquarters, Pokhara.
8. You have to **coordinate with ACAP Unit Conservation Offices** while performing your field research work.
9. You and your research **team have to strictly follow all rules, guidelines and social norms to keep in safety from COVID-19 while doing your fields work**.
10. Any dispute arose during the execution periods will be solved by mutual understanding.
11. Any unsolved disputes will be handled as per the existing law of Nepal government.

Thank you and wish you all the best.

Raj Kumar Gurung
Project Chief

CC:
NTNC-ACAP Unit Conservation Office
Ghandruk / Lwang / Manang / Jomsom / Lomanthang