Ecological Outlook of *Gazalina chrysolopha* in High Seasonal Hyperacute Panuveitis Prevalent Districts of Gandaki Province, Nepal

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A dissertation submitted

In partial fulfillment of the requirements for the award of the degree

of Master of Science in Zoology with special paper Entomology

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September, 2024

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Citation: Pathak, P. (2024*). Ecological Outlook of Gazalina chrysolopha in High Seasonal Hyperacute Panuveitis Prevalent Districts of Gandaki Province, Nepal* (MSc dissertation), Central Department of Zoology, Tribhuvan University.

Declaration

I hereby declare that the work presented in this dissertation "**Ecological Outlook of *Gazalina chrysolopha* in High Seasonal Hyperacute Panuveitis Prevalent Districts of Gandaki Province, Nepal**" has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

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Certificate of acceptance

This dissertation work submitted by Pratiksha Pathak entitled "**Ecological Outlook of *Gazalina chrysolopha* in High Seasonal Hyperacute Panuveitis Prevalent Districts of Gandaki Province, Nepal**" has been accepted as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper Entomology.

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Acknowledgments

I would like to express my sincere appreciation to my supervisor, Associate Professor Dr. Daya Ram Bhusal, Central Department of Zoology for his supervision and encouragement throughout the research process. I am thankful to Professor Dr. Kumar Sapkota, Head of Central Department of Zoology, T.U for providing essential consent and recommendations for this research. I respectfully acknowledge to Department of Forests and Soil Conservation for permission to conduct the research in different forest of Lamjung, Kaski, Gorkha, Parbat, Baglung, Myagdi and Syangja districts.

My sincere thanks to the Tribhuvan University National Priority Area Research Grant (TU- NPAR-078/79-ERG-01) of Tribhuvan University, Nepal.

My special thanks go to my friend Bhawana Pandey, Surya Devkota, Niten Bharati, Tsunami Thapa Magar and Janaki Pathak for supporting me throughout my research work. I am also thankful to all the field enumerators for helping me during my field work. Thanks to all the social respondents for their valuable time and information, they provided during the survey.

Special thanks to my family for their patience, understanding, and constant encouragement.

Thank you all for being an integral part of this academic endeavour.

Abstract

The moths are the insects which can coil their proboscis, primarily grey or brown color. They are mostly nocturnal and plant feeder. This study was carried out at seven different districts of Gandaki province from mid-August to December of 2023 using the light trap method, direct hand picking and sweeping net method for the extensive collection of moth. The adult, egg, larvae and pupa of *Gazalina* species were observed and collected. The specimens were collected in killing jar with cotton soaked in ethyl acetate. The aims of this study were to identify the microhabitats and host plants, relation of bioclimatic parameters in distribution of *Gazalina chrysolopha,* and medical importance of moth. The microhabitat of moth is specifically on host plant's leaves, bark, and walls of houses. The analysis shows the number moth were high in *Alnus* forest followed by mixed forest and household. The distribution of adult was high at the *Alnus* forest associated with human settlement sites followed by *Alnus* forest only. The relative humidity and temperature does not show strong relationship with abundance of moth. The body length and head capsule increases subsequently throughout the instars and brown hairs cover the body segment. The numbers of SHAPU patients and moths are highly correlated; showing high number of SHAPU patients where the number of moth was higher. The detailed study on the ecological behaviour of this moth can be beneficial to entomologist and medical faculties. This study presents the microhabitat of *G. chrysolopha* in selected sites of seven districts of Gandaki Province along with the factor related to distribution, larval growth and medical important.

Keywords: Pest, *Alnus nepalensis,* microhabitat, medical important

शोध सार

Moth कीराहरू हुन् जसले आफ्नो proboscis लाई घुमाउन सक्छन्, मुख्य रूपमा खैरो रङका हुन्छन् । तिनीहरू प्रायः निशाचर र बोट बिरुवा feeder हुन्छन् । गण्डकी प्रदेशका विभिन्न सात जिल्लामा २०८० को मध्य-भदौ देखि पुसम्म light trap, प्रत्यक्ष hand picking र sweeping net को प्रयोग गरी moth को व्यापक सङ्कलन गरियो । *Gazalina* प्रजातिका वयस्क, अण्डा, लार्भा र प्युपालाई अवलोकन गरी सङ्कलन गरिएको थियो । Ethyl acetate मा भिजाइएका कपास भएको killing jar मा नमूनाहरू संकलन गरिएको थियो । यस अध्ययनको उद्देश्य सूक्ष्म बसोबास र host बिरुवाहरू, *Gazalina chrysolopha* को वितरणमा bioclimatic मापदण्डहरूको सम्बन्ध, र कीराको चिकित्सा महत्व पहिचान गर्नु थियो । कीराको सूक्ष्म निवास विशेष गरी होस्ट बिरुवाको पात, बोक्रा र घरका भित्ताहरूमा हुन्छ । विश्लेषणले *Alnus* जंगलमा, मिश्रित वन र घर वरिपरि कीराको संख्या बढी रहेको देखाएको छ। वयस्कको वितरण मानव बसोबास स्थलहरु संग सम्बन्धित *Alnus* वनमा उच्च थियो त्यस पछि *Alnus* वनमा मात्र । सापेक्षिक आर्द्रता र तापक्रमले कीराको प्रचुरतासँग बलियो सम्बन्ध देखाउँदैन । पछिका इन्स्टारहरूमा शरीरको लम्बाइ र टाउकोको क्याप्सुल बढ्दै जान्छ, भने खैरो रौंले शरीरको खण्ड ढाक्छ । SHAPU बिरामीहरूको संख्या र moth हरूको संख्या उच्च रूपमा सम्बन्धित छन, जहाँ सापु बिरामीको उच्च संख्या थियो त्यहाँ कीराको संख्या पनि बढी थियो । यस moth को पारिस्थितिक व्यवहारको विस्तृत अध्ययनले कीटविज्ञानी र चिकित्सा संकायहरूको लागि लाभदायक हुन सक्छ । यस अध्ययनले गण्डकी प्रदेशका सात जिल्लाका छनोट गरिएका स्थानहरूमा *G. chrysolopha* को सूक्ष्म बसोबास र वितरण, लार्भा वृद्धि र चिकित्सा महत्वसँग सम्बन्धित कारकहरू प्रस्तुत गर्दछ ।

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List of abbreviations

ACA- Annapurna Conservation Area

GPS- Global Positioning System

RH- Relative Humidity

SHAPU- Seasonal Hyper Acute Pan-Uveitis

Introduction

1.1 Background

The moths (Order; Lepidoptera) are only group of insects that have scales covering their wings, with few exceptions (Regier et al., 2009). They can coil their proboscis which differ them from other insects. They can be distinguished from butterfly and other insects through observation of antennae (typically threadlike or feathery). They have most part relatively dull wing colors but there are many species with spectacular colors and patterns, shape and size also vary according to species. They are mainly nocturnal attracted to light and plant feeding although some species are diurnal (Wahlberg et al., 2013). They serve as prey for several species of rodents, birds, and bats. They are a significant component of terrestrial ecosystems (Bates et al., 2014). Moths are holometabolous insects with complete metamorphism during lifecycle which occupy wide range of habitat. The effective ecological indicators for the preservation of biodiversity can be obtained by tracking changes in the patterns of moth distribution and abundance in a given area (Dennis et al., 2019).

The family Notodontidae had almost 3,800 species around the world (Hampson, 1892; Miller, 1992). This family of moth is widespread throughout the planet, but it is the most prevalent in tropical regions, particularly in the New World (Miller, 1992). Majority of the species in this family have long wings that are folded across the rear part of the body while at the rest, they hardly ever exhibit vivid colors, preferring to be primarily grey or brown instead (Grimaldi and Engel, 2005). Three species (*tranversa*, *apsara*, and *chrysolopha*) of the *Gazalina* moths are added to the catalog of Nepal's moths (Smith, 2010; Poel & Sajan, 2022). The *Gazalina chrysolopha* was observed in Godawari, 1,600 meters south-east of Kathmandu (Haruta, 1993). It was also discovered in eastern Nepal's Dagchu (2,880 m) and Jiri (2,340 m) during another expedition (Haruta, 1994). The distribution has been limited to the mid-mountain districts; there is no information available from the Terai region. These moths peak in number in September and the first week of October, however they emerge in the middle of July (Khanal & Shrestha, 2022). The primary habitat of *G. chrysolopha* are the northwest Himalaya and Sikkim (Hampson, 1892). The *G. chrysolopha* is mainly found in Gandaki Province preferring odd monsoon season. The warmer temperatures, more frequent rainstorms, and higher levels of humidity create ideal conditions for the hatching of the white moths' un-hatched

eggs (Gurung et al., 2021). Its larvae are a significant pest that severely defoliates the oak forests (Rahman & Chaudhry, 1992). It has been discovered to significantly defoliate the shade tree (*Alnus nepalensis)* of huge cardamom, exposing the undergrowth to excessive sunlight, cold, and other climatic conditions (Srivastava, 2003).

The male *Gazalina chrysolopha* has a white head and thorax, black legs with black shaft antennae. Black and white with fringed segments is seen on the abdomen. Male and female moths are similar, except the females have a larger fulvous, less fringed abdominal segment, and an anal tuft with barbed setae. Females have narrower abdominal white segmental fringes. In order to safeguard the eggs, the female released anal tuft setae, which are easily carried by the wind (Srivastava, 2003; Srivastava & Mukhopadhyay, 2006). The life cycle of *G. chrysolopha* is annual and larva show aggregation and nocturnal behavior. Annual life cycle restricted the fast multiplication assuring natural enemies to breed a large numbers and controlling the pest population (Wali M.IsmailChaudhry, 1992). The larvae often lack hairs, however they could have spines, humps or tubercles (Gaston et al., 1995). Most of the gregarious feeders are solitary, and processionary moths, Thaumetopoeinae, are the most common examples. Notodontidae larvae have chemical defenses (cyanic acid, formic acid, and other ketones) that are uncommon in other Lepidoptera (Blum et al., 1981). The adult females of certain genera of Notodontidae receives a poisonous fluid from epidermal glands through their irritating barbs, which serve as shield to eggs from harm. During monsoon season they become airborne and trigger dermatitis outbreak in human population (Rothschild et al., 1970).

1.2 Statement of problems

Gazalina chrysolopha is the major defoliator of the *Alnus nepalensis*. The larvae of this moth severely damage the leaves of the host plants causing declination in the number of plants population at specific area. The larvae are voracious feeders feeding on the whole leaf of a plant causing death of the plant due to deficiency of food in plants. The female adult contain yellow tuft on the abdominal tip which may cause irritation and itching with contact in human skin and eyes. The adult moth's hair particles is suspected as the major cause of eye diseases Seasonal Hyper Acute Pan Uveitis (SHAPU) occurring only in Nepal (Kharel et al., 2020; Gurung et al., 2021; Upadhyay et al., 2021). There is huge research gap in the microhabitat and host plant preference by *Gazalina chrysolopha.*

1.3 Objectives

1.3.1 General objectives

The general objective of the study is to explore ecological outlook of the *G. chrysolopha.*

1.3.2 Specific objectives

The specific objectives of the study are:

i. To identify the microhabitat and host plants of *Gazalina chrysolopha*
ii. To determine the feeding attributes of *G. chrysolopha*
iii. To find the relation of moth abundance with bioclimatic parameters
iv. To find the relation of SHAPU records with adult abundance

1.4 Research question(s)

- Is there any specific microhabitat selected by *G. chrysolopha*?
- Is *G. chrysolopha* medically important insect?

1.5 Significance of the study

The *Gazalina chrysolopha* is the major defoliator of *Alnus nepalensis* and the major factor causing SHAPU diseases related to eyes. This study investigate the microhabitat and host plant of *G. chrysolopha*, relation with bioclimatic parameters, feeding attributes, relation with SHAPU records and structure of setae of *G. chrysolopha*. The microhabitat selection and host plant enhance the biology of this moth. The study related to its larval growth enhances the control and prevent the outgrowth of these moths. The study of structure of setae contributes the important data for establishing a foundation for future studies. Overall, the study has significant implications for ecological and national health programs, for the development of effective preventative and control techniques, as well as educational campaigns to raise awareness of the moth.

1.6 Limitation of the study

- The comprehensive covering of large area was not possible due to physical challenge.
- Lack of sufficient references based on the microhabitat and host plant of *Gazalina chrysolopha*.

2. Literature review

2.1 Microhabitat and host plants of *Gazalina chrysolopha*

Haruta (1993) identified *Gazalina chrysolopha* at Godawari, 1,600 south-east of Kathmadu.

Haruta (1994) discovered G. *chrysolopha* in eastern Nepal's Dagchu and Jiri during another expedition.

Srivastava (2003) showed an experimental study on leaf consumption and utilization was carried out using the third, fourth, and fifth instars larvae of *G. chyrsolopha*. The early instar larvae munched on debris/very fragile leaves (in the lab) discovered at the base of the A. *nepalensis* tree. Presence of lacewings in the fields preyed on the eggs of *G. chrysolopha* was observed. Additionally, ants (*Crematogaster* sp.) attack, maximum parasitism was seen in the insect's larval stages. Tachinid fly larvae parasitized late-instar *G. chrysolopha* larvae.

Sondhi & Sondhi (2016) found 248 species of moths during surveys conducted over 40 nights in Dehradun and Mussoorie in Dehradun District and Devalsari in Tehri Garhwal District in Uttarakhand.

Dewan et al., (2022) found ten *G. chrysolopha* individuals on August 28 and 29, 2021, in Mustang district (28.770°N and 83.727ºE, 2285 m). Additionally, the district is a portion of a trans-Himalayan zone that is a part of Annapurna Conservation Area (ACA). In the daytime, moths were seen resting near light sources on a wall, a window, and the ground.

Khanal & Shrestha (2022) reported *Gazalina chrysolopha, G. apsara, G. transversa* are the three species found in Nepal. *Gazalina chrysolopha* is the widely distributed species attracted to the white light source. The *G. chrysolopha* is distributed in the mid hilly area, with no record of distribution in Terai region.

2.2 Feeding attributes of *G. chrysolopha* relation

Brues (1920) explained in Lepidoptera (butterfly and moths), the larvae are the main feeding stage of each species and are usually restricted to feeding on one or a few related plants genera.

Phaloura & Singh (1991) observed *Gazalina chrysolopha* was the major folivores that defoliated *Alnus nepalensis* in different seasons. Earlier reports also labeled *G. chrysolopha* as the major folivores of *A. nepalensis.*

Wali M.I.Chaudhry (1991) explained in 1198 *G. chrysolopha* caused serious defoliation on oak forest over an area of 1600 hectar in Bagh Forest Division of Azad Jammu and Kashmir. Adult emerging around July and August month, larval stage round 10–11.5 months. Larval stage is the damaging stage of this moth. Pupal stage occurs for 1–2 months. The life cycle of *G. chrysolopha* is annual.

Rahman & Chaudhry (1992) described *G. chrysolopha* larvae as major defoliators, by the study of its life cycle on oak trees in Pakistan. Explain the period of egg, larva, pupa and adult. The adult moth emerged from mid- July to mid-August and laid clusters of eggs on the dorsal surface of the leaves. Egg hatched from mid-august to mid-September, show nocturnal and processionary behaviour. The life stage of larvae occurred for10–11.5. The pupation took palce for 1–2 months around June and July month.

Raman (1998) in his article "Outbreak of *Gazalina chrysolopha* and defoliation of *Alnus nepalensis* in eastern and Central Bhutan" mentioned about some larval and pupal parasitoids of *G. chrysolopha* attacking *Alnus* tree in Bhutan.

Srivastava (2003) showed an experimental study on leaf consumption and utilization was carried out using the third, fourth, and fifth instar larvae of *G. chyrsolopha*. The early instar larvae munched on debris/very fragile leaves (in the laboratory) discovered at the base of the A. *nepalensis* tree.

2.3 Relation with bioclimatic parameters

Srivastava (2003) showed an experimental study on leaf consumption and utilization was carried out using the third, fourth, and fifth instars larvae of *G. chyrsolopha*. Maximum and minimum temperatures from three years, year 2000 shows negative correlation with the pest population whereas pest population was influenced by only minimum temperature in years 2001 and 2002. Day cloud cover and Relative humidity had a non-significant effect on the population of *G. chrysolopha*.

2.4 Medical importance of *G. chrysolopha*

Battisti et al.,(2017) described the Asian species of *Gazalina* carry setae in the adult females which may be released incidentally when attracted to lights, generating serious skin reactions and even blindness in children who come in contact with them. The larva of *G. chrysolopha* have not been studied so could not suspect the occurrence of setae. The dorsal structure of larva is similar to the larva of Thaumetopoinea spp.

Manandhar et al., (2018) suspect *G. chrysolopha* as the prime pathogen carrier for the eye diseases SHAPU. Twenty- three percent of patients were reported with the history of contact with white moth. Fine brown hairs were present in 5 patients. Patients from 25 different districts, 15 from Kathmandu valley, followed by 8, 7, and 6 respectively from Dolakha, Kavreplanchowk and Sindhupalchowk districts. Highest from hilly region (18%) followed by terai region (18%), and mountainous (15%) region respectively.

Upadhyay et al.,(2021) conclude direct physical contact with moths was associated with significantly increased odds of SHAPU cases. The months of *G. chrysolopha* emerging show the highest number of SHAPU patients significantly.

Khanal & Shrestha (2022) reported the *Gazalina* specis has toxic hairs suspected to cause severe eye infection on the contact human with eyes. *Gazalina chrysolopha, G. apsara, G. transversa* are the three species found in Nepal. *G. chrysolopha* is the widely distributed species attracted to the white light source. The *G. chrysolopha* is distributed in the mid hilly area, with no record of distribution in Terai region.

3. Materials and methods

3.1 Study area

The field study was carried out in the seven districts of Gandaki province (Gorkha, Lamjung, Kaski, Syangja, Parbat, Baglung and Myagdi). The study was done on the selected sites of seven different districts of Gandaki Province. The districts have different climatic variation and elevation whereas the primary host plant nearby the city was similar *Alnus nepalnensis* (Figure 1).

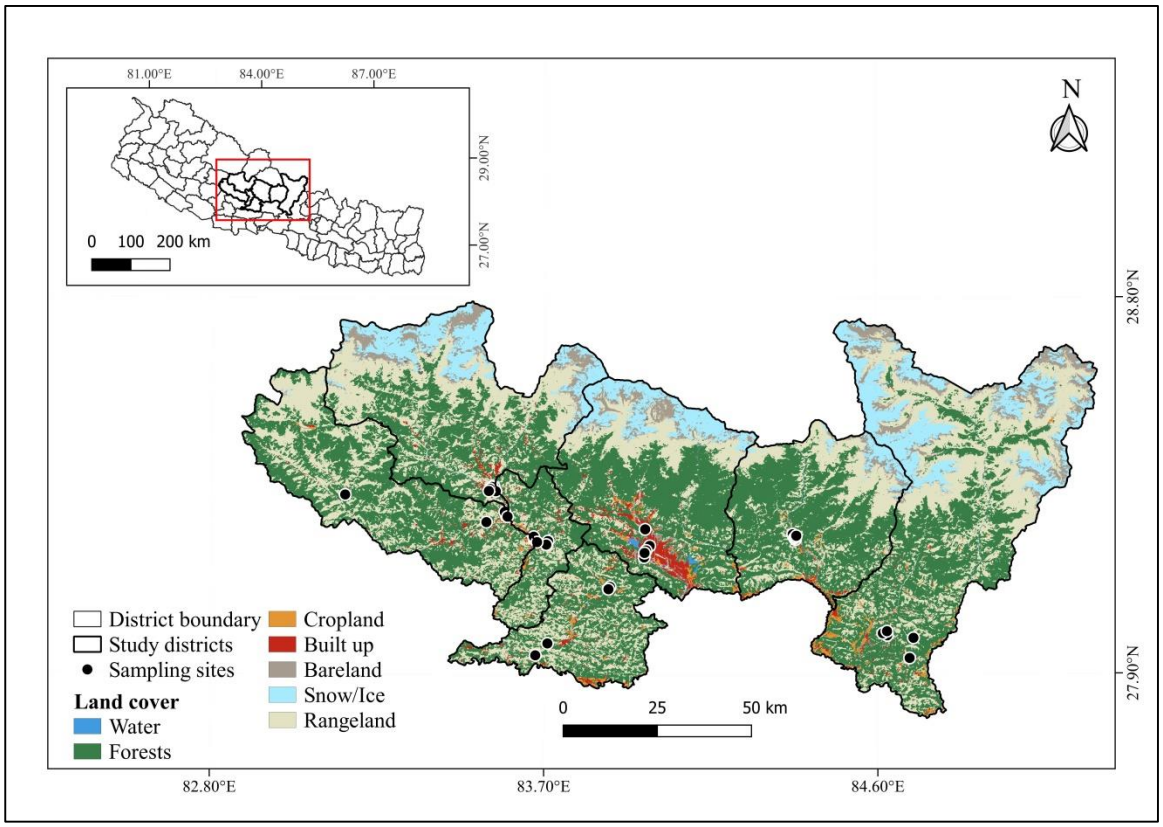

Figure 1: Map of the study area

3.2 Field design

In this study, seven districts were selected based on the presence of SHAPU patients in the previous years. Based on the local information, literature review and previous medical records about SHAPU outbreak in Gandaki Province, sampling sites for the moth collection was ascertained. Thereafter, the selected areas were further categorized as SHAPU recorded site based on hospital information. Five sites were studied in each district in August to December of 2023 for the sample collection.

3.3 Collection of moth

The moths were collected from August to December during the year 2023 by installing the light trap method (mercury vapour light traps method) (Sondhi & Sondhi, 2016). The light trap was continued for 7 days nights at 7–11 pm within selected sites. The specimens were collected in a killing-jar with cotton soaked in an ethyl acetate and later stored in tracing paper envelopes. Additionally, hand picking and sweeping net methods at nearby household, forest area and agricultural site were done for extensive collection. Safety measures and precautions were followed by wearing goggles, gloves, face mask, surgical cap, for the prevention from moth's setae contact. The collected sample species were kept in paper envelopes with preservative for further sorting and identification, based on their morphology features.

For the identification of microhabitat, and relation with specific climatic factors of moth, following ecological parameters were collected during the field visits:

i. Local temperature (°C) and relative humidity (RH %) (Hygrometer).

ii. GPS point of each sites of moth collected (etrex 10 GARMIN).

iii. Surrounding vegetation, flower patches nearby, forest distance from trapping house, presence or absence of suspected host plant nearby (i.e., *Alnus nepalensis)* and its distance from collected households.

3.4 Preservation and species identification

The collected samples were carried to the laboratory of Central Department of Zoology. The specimens were sorted based on the morphological characters and pinned on the entomological box. The naphthalene balls were added as preservative. *Gazalina chrysolopha* (i.e., fore wings having indistinct sub basal black line, a streak along median nervure, and two slightly waved median oblique lines beyond which veins are black, the thorax and collar are tinged with fulvous, where the female having golden brown tuft of spines on their abdomen) were separated from other moth species from the samples. The collected moths were observed in Stereoscopic microscope using taxonomic keys for identification of *G. chrysolopha* as suggested from Nepal and adjacent countries (Haruta, 1993; Sondhi & Sondhi, 2016). The collected specimens of *G. chrysolopha* are deposited and preserved in the museum of Central Department of Zoology, Tribhuvan University.

3.5 Data processing and analysis

Data obtained from field were organised and analysed in Microsoft Excel 2010. The regression analysis was done for relation the bioclimatic factors and abundance of moth using R-studio. The Microsoft Excel 2010 was used to identify the correlation between number of moth and number of SHAPU patients.

4. Results

4.1 Microhabitat of *G. chrysolopha*

The microhabitat preference by adult *G. chrysolopha* is generally any objects near light source. The different phases of moth's life cycle have different microhabitats. The specific microhabitats of moth are tabulated as below:

Table 1: Frequently observed microhabitats of *G. chrysolopha*

Phases	Microhabitat
Egg	Dorsal side of *Alnus* plant leaf, walls of the house, street light poles,
Larva	Dorsal side of leaf and bark during dawn, dusk and night, base of the host the host plant during day time
Pupa	In the soil at the base of host plant *A. nepalensis*
Adult	Any suitable sites majorly on the wall of building and plant near white light source

4.1.1 Oviposition sites of moth

The female *G. chrysolopha* lays egg after 5-6 days of emergence. There were a significant proportion of moth eggs laid on the dorsal surface of *Alnus* plants (Figure 2B). A very few plants other than *Alnus* were also observed with cluster of eggs (Figure 2C). The numbers of clusters were laid on the wall of houses, street light poles as well. The numbers of egg cluster were higher in the *Alnus* plants leaves as the numbers of adult were also present near the *Alnus* forest. The eggs lay on the walls of houses, street light poles were infected by algae and fungi (Figure 2A). The eggs laid on the leaves were only thriving and ready to hatch as larvae.

Figure 2: Oviposition; (A) on walls of houses (B) on the dorsal side of *A. nepalensis* leaf (C) on the bamboo plant (D) on the umbrella

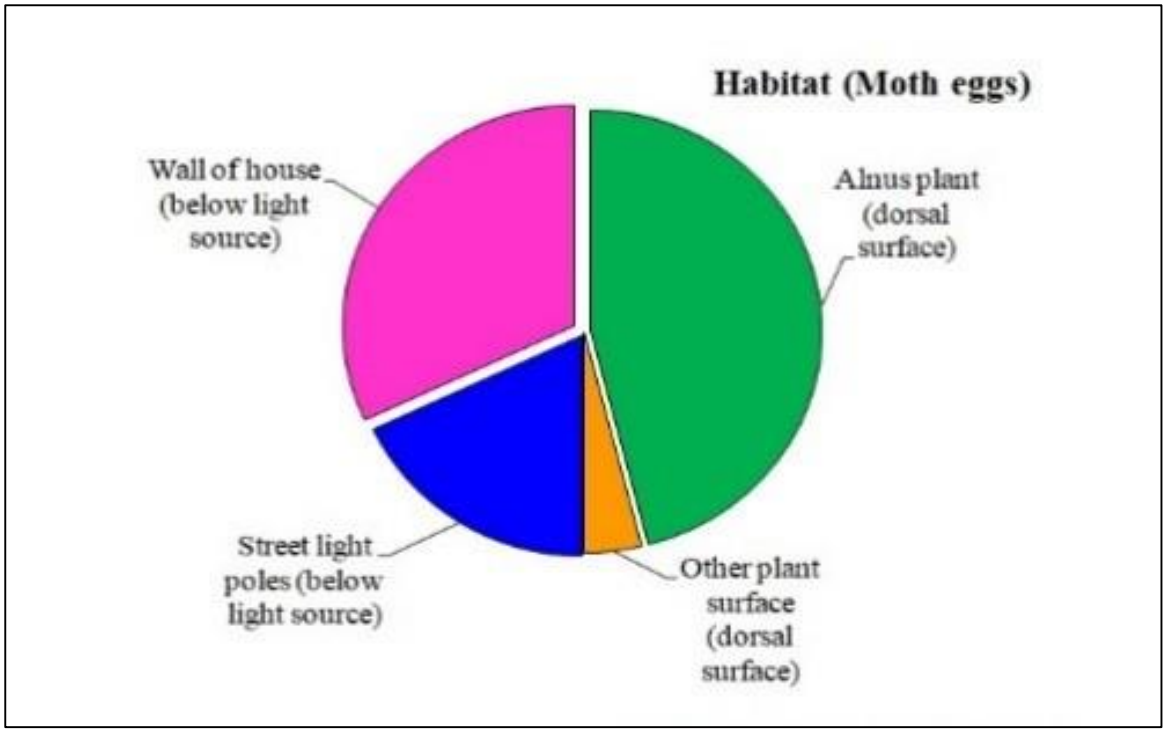

Figure 3: Pie chart showing different microhabitat of eggs

4.1.2 Microhabitat of larvae

Gazalina chrysolopha larvae were found on the host plant, *Alnus nepalensis*. After 60 days of oviposition, the larvae emerge on the base (Figure 4C) and the bark of the host plant (Figure 4A). The very few larvae were observed on the dorsal side of leaf (Figure 4A) and ground near host plant. The *G. chrysolopha* larvae have five instars, exhibiting nocturnal habits, feeding at night and resting throughout the day on the base of the host plant. They spend the day resting at the base of the host plant. As they proceeded in a trail, leaves white lining mark on the host plant's bark. The first and second instar feed solely on *A. nepalensis*, whereas the third, fourth, and fifth instar feed on secondary host plants as well.

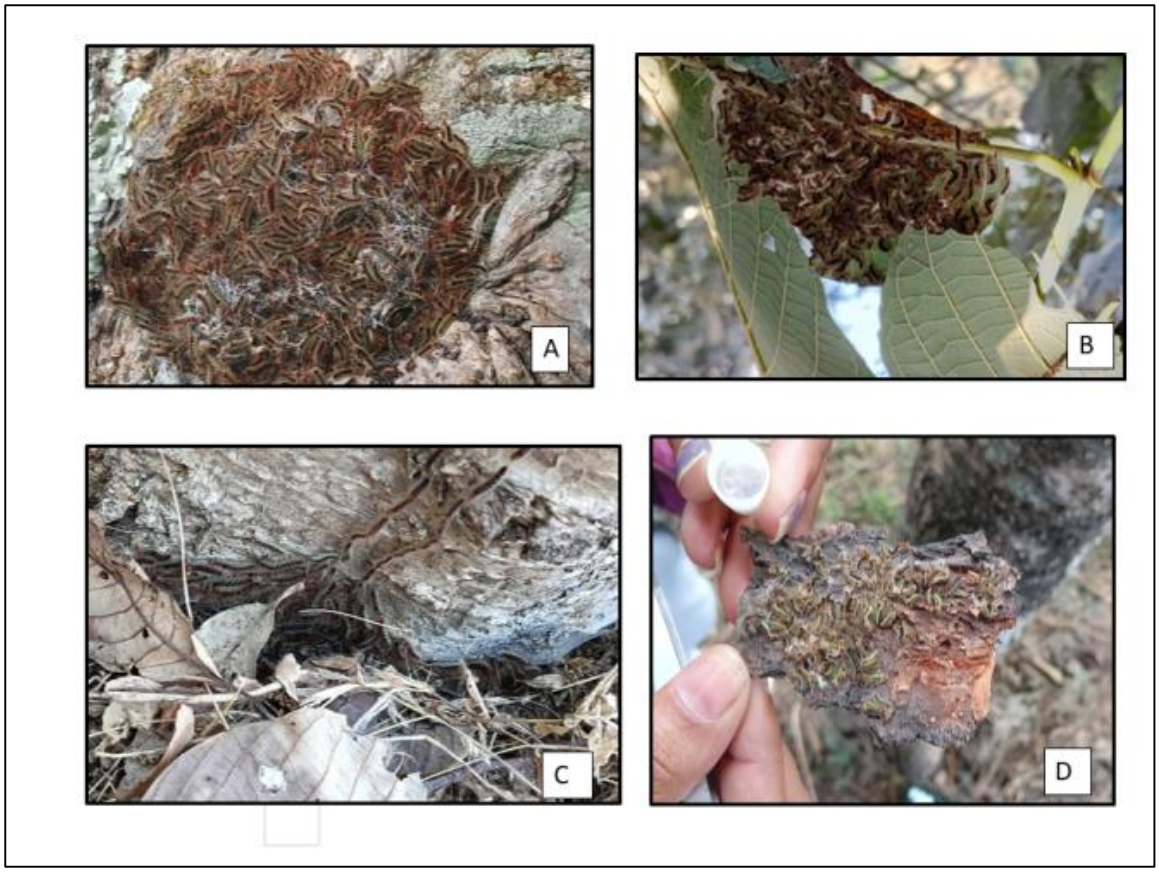

Figure 4: Larvae in different microhabitat; (A) bark of the host plant (B) dorsal side of host plant (C) base of the host plant (D) beneath the bark of plant

4.1.3 Microhabitat of pupa

The pupal, an immotile stage were found in the soil at the base of *A. nepalensis* involves fifth instar larvae that cover themselves in silk, forming a cocoon. The cocoons were white in the initial phase but later turned reddish-brown, while the actual pupae are dark brown. The color change may indicate developmental stages or environmental factors. The pupa (Figure 5) is the non- feeding stage of the moth life cycle.

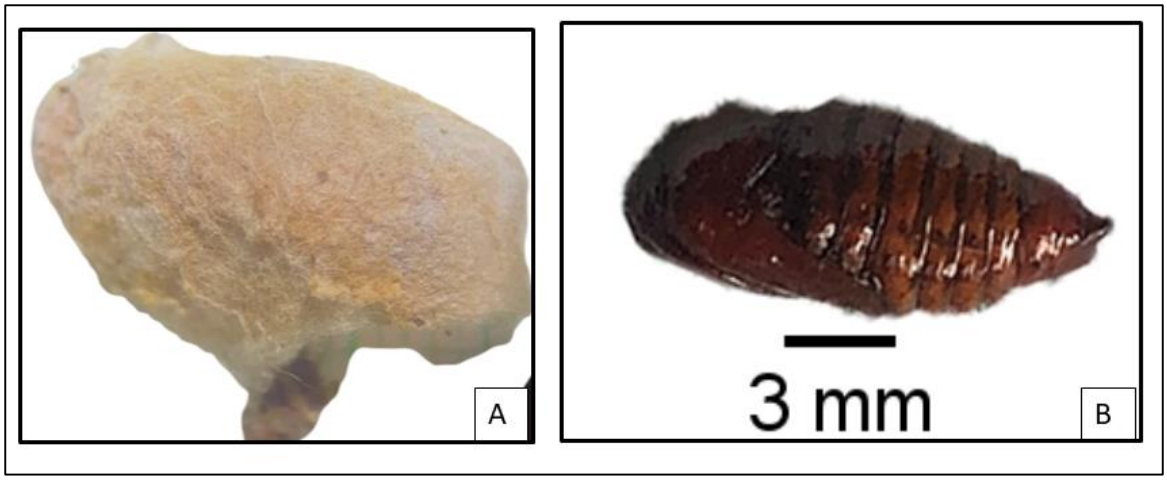

Figure 5: Pupa of *G. chrysolopha;* (A) inside cocoon (B) outside the cocoon

4.1.4 Microhabitat of adult *G. chrysolopha*

The *Gazalina* moth has no site-specific microhabitat preference; rather it rest at any suitable sites, majorly on the walls of buildings (Figure 6C) and on the leaves of the plants near light source. It seems, *G. chrysolopha* oviposit generally close to the light source (indoor or outdoor). The eggs are pale yellow and deposited by female adults (Figure 7A) in a mass cluster with gelatinous coverings on them for protection against fungi, bacteria, and predators. The highest percentages of adults were found in outdoor microhabitat forests followed by human settlements, and least commonly found in agricultural land. Adult moths (Figure 8) were observed resting on the walls of the houses, leaves, and objects (e.g., a wall, holding board, pole, cloths, etc.) near the light source. Generally, more adults were found in microhabitats with higher intensity light as they are attracted to the white light source.

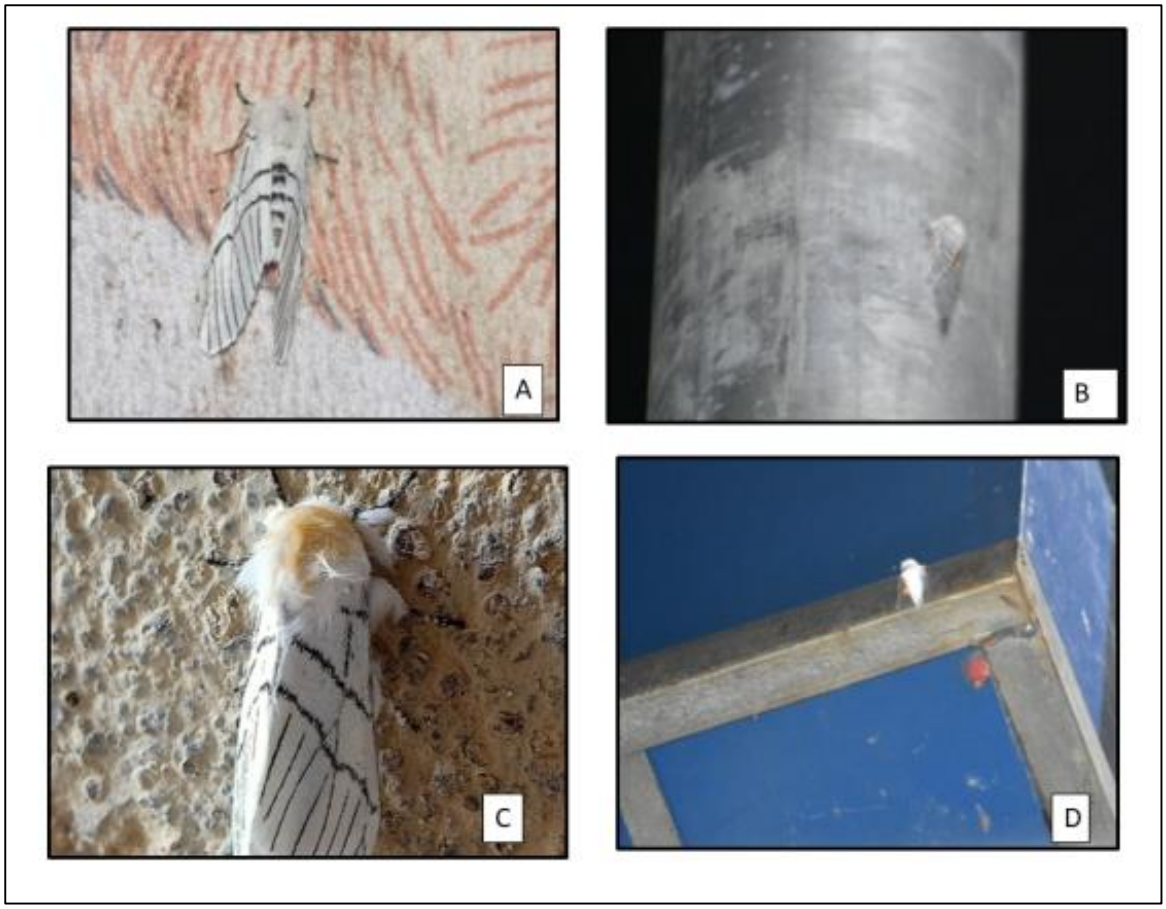

Figure 6: Microhabitat of adult moth; (A) on the cloths (B) on the street light poles (C) on the walls of house (D) below the light source

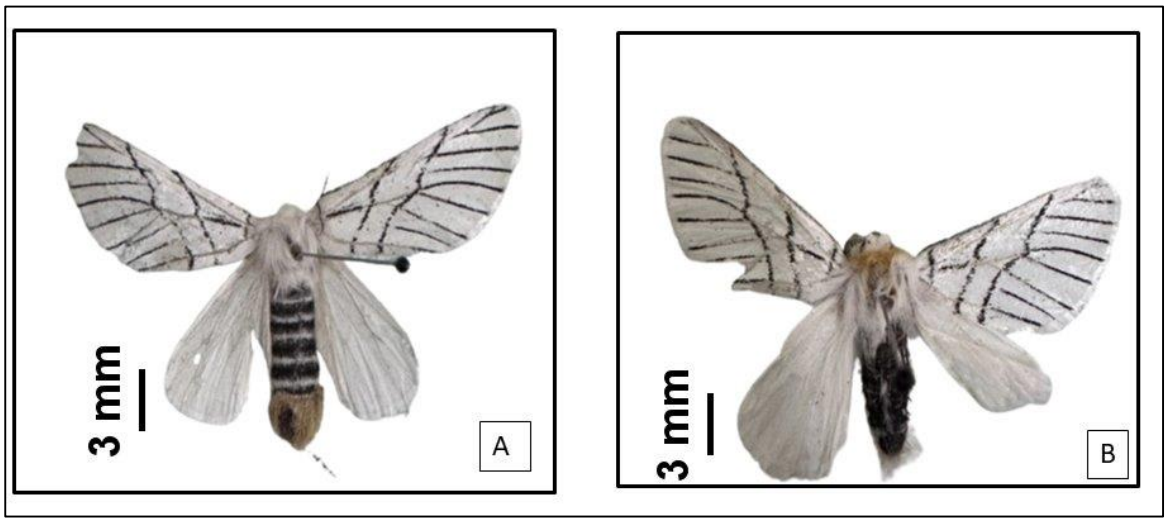

Figure 7: Adults of *Gazalina chrysolopha;* (A) Female (B) Male

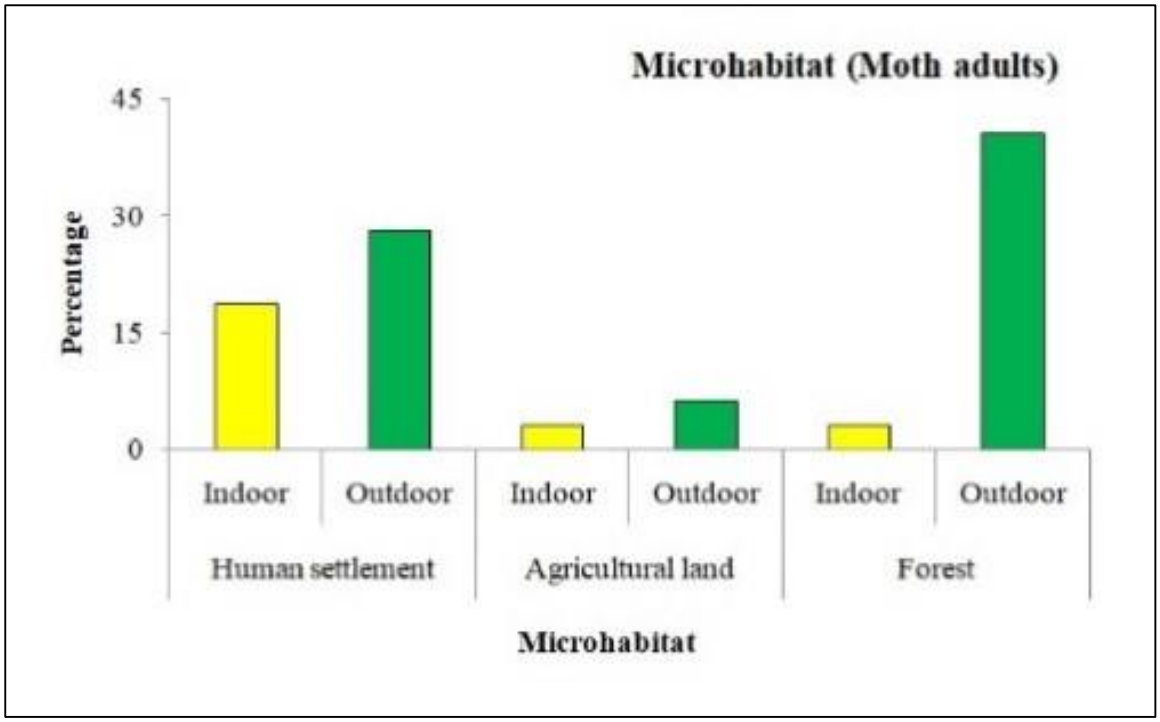

Figure 8: Abundance of adult in different microhabitats of adult moths

4.2 Host plants

The egg, larvae and adults are observed in the *Alnus nepalensis* and other plants nearby the *A. nepalensis* (Figure 9A). The eggs and larvae are on the dorsal side of leaves and bark of the host plant. The number of moth was higher in the *Alnus* forest nearby the human settlement. The graph (Figure 10) shows that human habitats associated with *Aln*us plants have a positive effect on the number of moths observed in the field. Mainly, *A. nepalensis* is the primary host, but in some areas, *Castanopsis* sp. and *Cinnamomum camphora* were also found as the primary hosts of this moth. After, damaging the primary host larvae moves to the nearby plants.

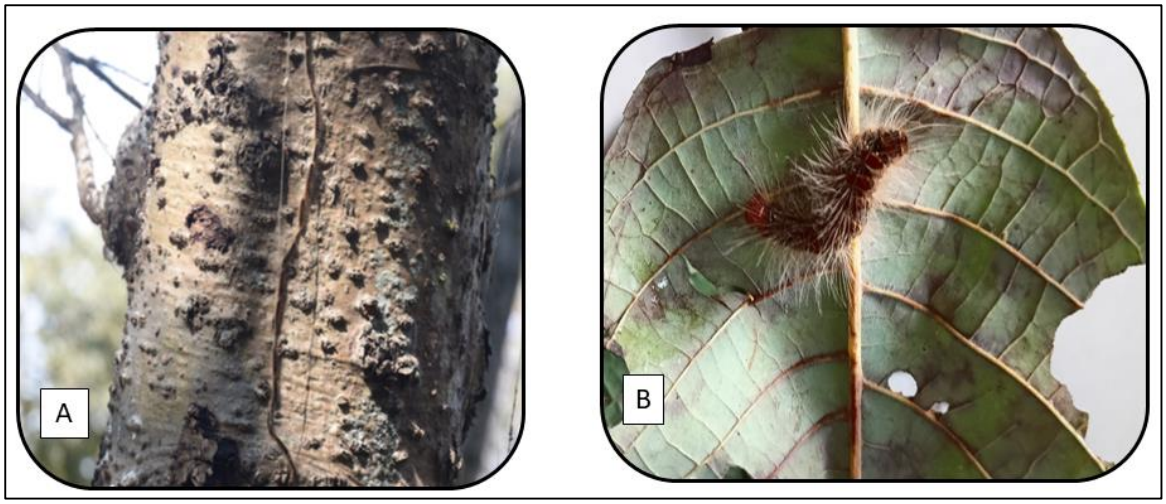

Figure 9: Host plant (*Alnus nepalensis*); (A) Bark (B) Leaf

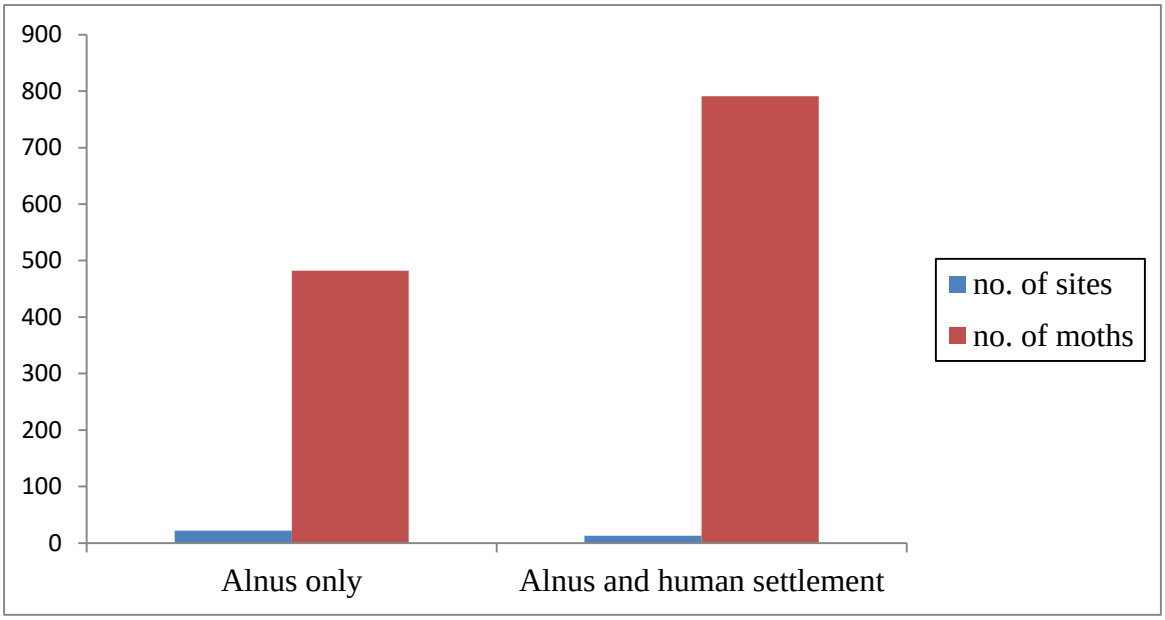

Figure 10: Graph of *Alnus* only and with human settlement and moth distribution

4.3 Feeding attributes of larvae of *G. chrysolopha*

The life cycle of *G. chrysolopha* has four stages: egg, larva, pupa, and adult. The egg laid in mid-September hatched between 45–60 days under a favourable environment. There are five instars in the larval phases of this moth. The larvae move upward during the night and feed on leaves of the host plants and move downward during the day, resting on the base of the host plant. The first instar and second instar larvae were collected from the field and reared at the entomological laboratory of Central Department of Zoology. The weight of larvae was taken in every 24 hours and physical parameters were taken 4 times a day.

4.3.1 First instar larva

The first instar larvae were found at the base of the host plant on the litter and new leaves of *A. nepalensis*, which were light brown in color during emergence, later turned dark; head brown-red, and the body covered with thin, light brown hair. They fed on leaves found near the base of the host plant. Body segments and legs are small, less developed and there are few setae present in the abdominal segments. The first instar (Figure 13A) moults into the second within 26–30 days.

4.3.2 Second instar larva

The feeding behavior of the second instar larvae becomes vigorous, which increases the length, dark brown color of the body, legs, and number of setae. The reddish socket, like humps are present in the dorsal abdominal segment. The second instar larva climbs trees and feeds on the leaves of the host plant, *A. nepalensis.* Unfortunately, the second instar died in the laboratory due to environmental factors. The second instar (Figure 13B) converts into the third instar within 30–35 days in a natural environment.

4.3.3 Third instar larva

The second instar larvae from a natural habitat were collected in the laboratory. The second instar larva moults to the third instar approximately within 30–45 days in the laboratory under room temperature. The third instar larvae become large in length and darker in color with long brown hair covering the tuft. The head capsules become large and deep red in color, third instar larva are active feeders and forages on the leaves of the host plant. The third instar larvae are darker and more developed in body length, legs, and colour. The number of setae increases and causes irritation and itching in contact with human skin and eyes. The third instar larvae (Figure 13C) moult to fourth instar within 40–45 days.

The third instar larvae have a significantly higher consumption rate compared to the second instar. The second instar larvae are smaller and less voracious feeders than the third instar larvae. The body length and body weight of the third instar is higher comparatively. The third instar larvae show a higher body biomass than the second instar larvae. The growth rate of the second instar larvae is higher than that of the third instar (Figure 12). The developmental phase of the third instar is also higher. The third instar larvae exhibit greater conversion efficiency than the second instar.

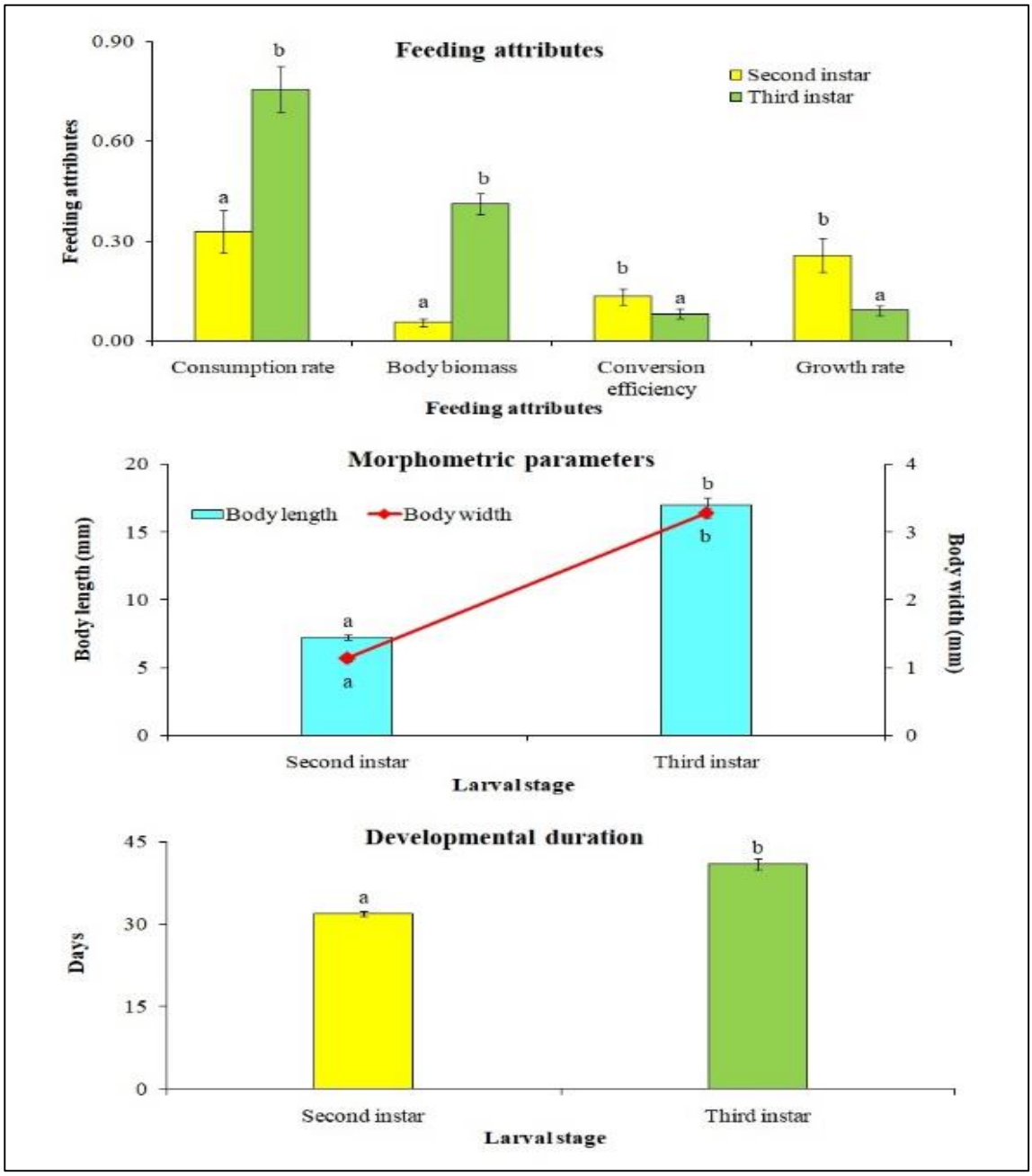

Figure 11: Feeding attributes and developmental duration of the second and the third instar larvae

4.3.4 Fourth instar larva

The third instar moults into the fourth instar within 40–45 days at room temperature. The fourth instar larva is a vigorous feeder, damaging the whole leaf of the host plant. The length and weight increase along with the head capsule, which has a darker body color. The body is covered with longer brown hair than previous instars. The fourth instar (Figure 13D) died during experiment; fourth instar larvae were again collected from natural habitat and reared.

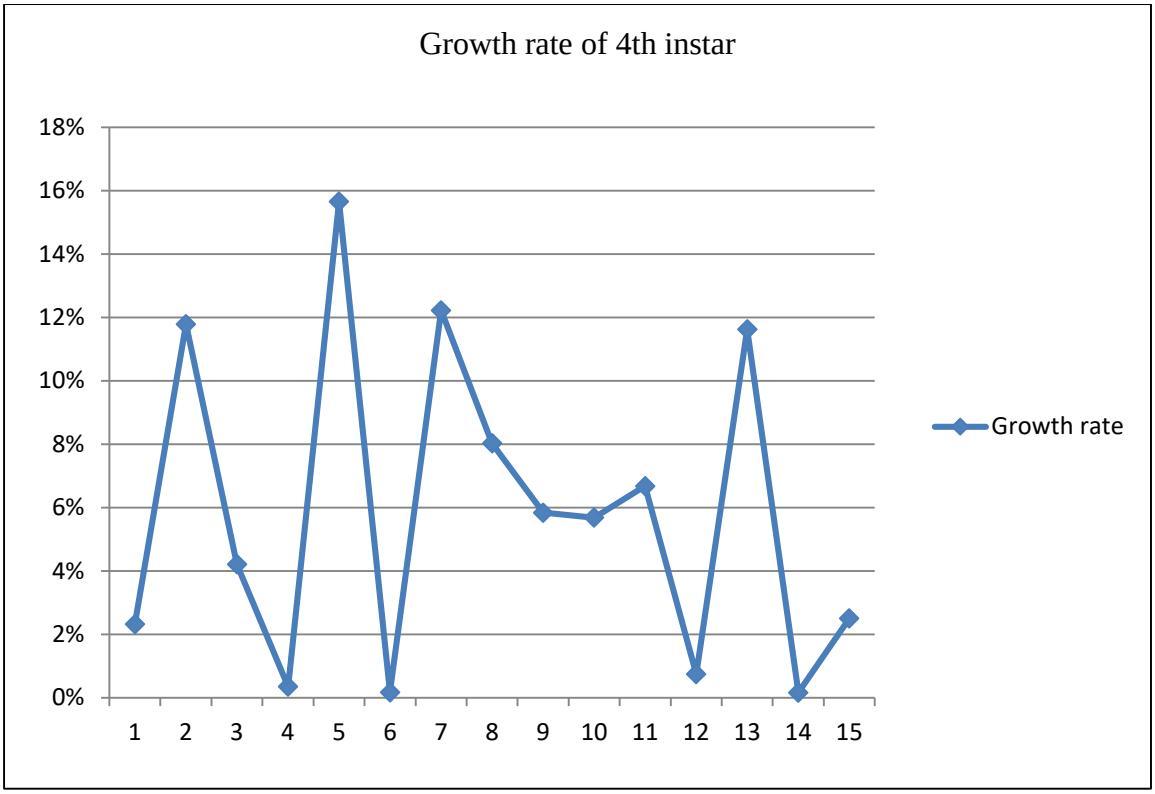

Figure 12: Growth rate of fourth instar larva within 15 days

4.3.5 Fifth instar larvae

The fourth instar larvae molts into fifth instar within 30–45 days. The fifth instar larvae (Figure 13E) are well developed and contain large numbers of setae on the dorsal side of the abdominal segments. The setae are long in length and dark reddish brown in colour.

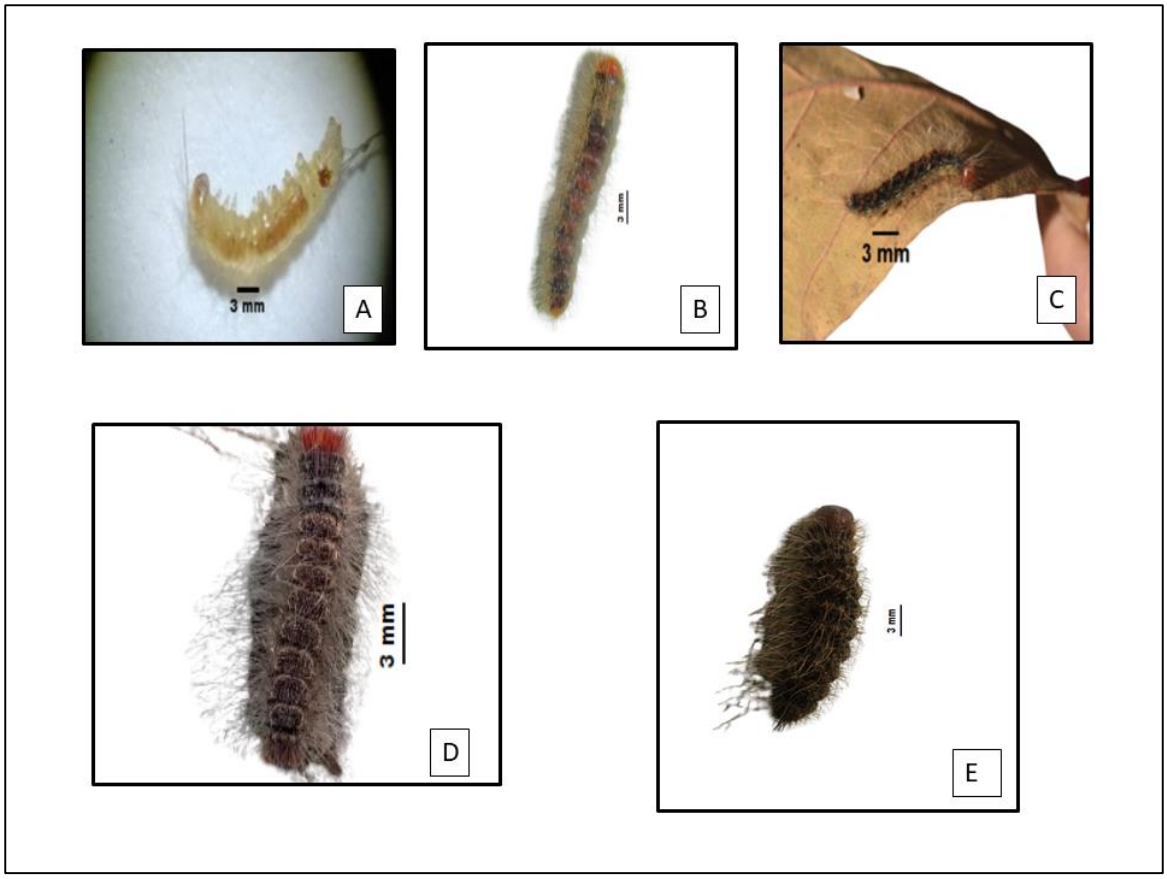

Figure 13: Different phases of larva; (A) first instar (B) second instar (C) third instar (D) fourth instar (E) fifth instar

4.4 Relation of moth abundance with bioclimatic parameters

The highest temperature is observed in Syangja, while the highest relative humidity is in Parbat. The lowest temperature and relative humidity are in Myagdi. Kaski has the highest number of moths and the highest number of SHAPU cases. Other districts have fewer moths and SHAPU cases, with Parbat having the fewest SHAPU cases despite having a relatively high number of moths. The regression analysis between dependent factor (number of moth) and independent factors (temperature and relative humidity), shows there is not significant relationship between dependent and independent factors (Table 2, 3). The analysis shows higher p- value and lower R square value explaining the data are not statistically significant.

Table 2: Regression analysis between adult abundance and relative humidity

Regression Statistics					
Multiple R	0.25				
R Square	0.06				
Adjusted R Square	-0.12				
Standard Error	169.92				
Observations	7				
ANOVA					
	df	**SS**	**MS**	**F**	**Significance F**
Regression	1	9858.11	9858.11	0.341	0.58
Residual	5	144367.31	28873.46		
Total	6	154225.42			
	Coefficients	Standard Error		t Stat	P-value
Intercept	-40.902	410.008		-0.1	0.92
RH(X)	3.38	5.799		0.584	0.584

Table 3: Regression analysis between adult abundance and temperature

Regression Statistics					
Multiple R					0.12
R Square					0.01
Adjusted R Square					-0.18
Standard Error					174.19
Observations					7
ANOVA					
	df	SS	MS	F	Significance F
Regression	1	2508.65	2508.7	0.08268	0.78
Residual	5	151716.77	30343		
Total	6	154225.42			
		Coefficients	Standard Error	t Stat	P-value
Intercept		-91.60	1001.42	-0.09	0.93
Temperature(X)		10.32	35.90	0.2875	0.78

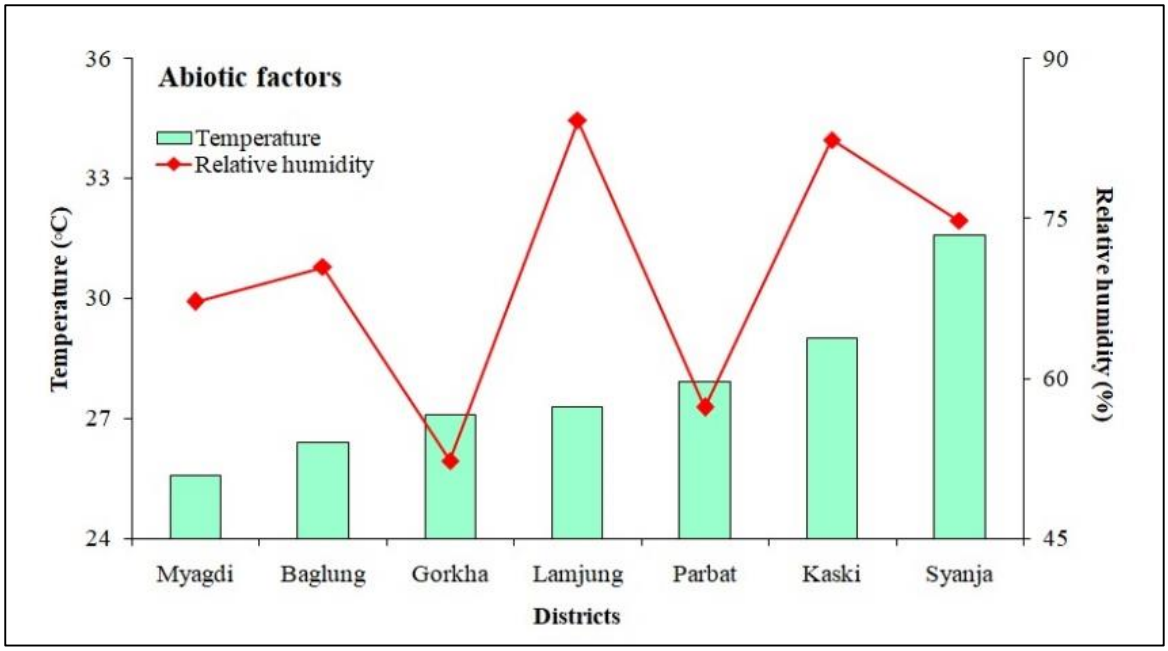

Figure 14: Relation of relative humidity and temperature

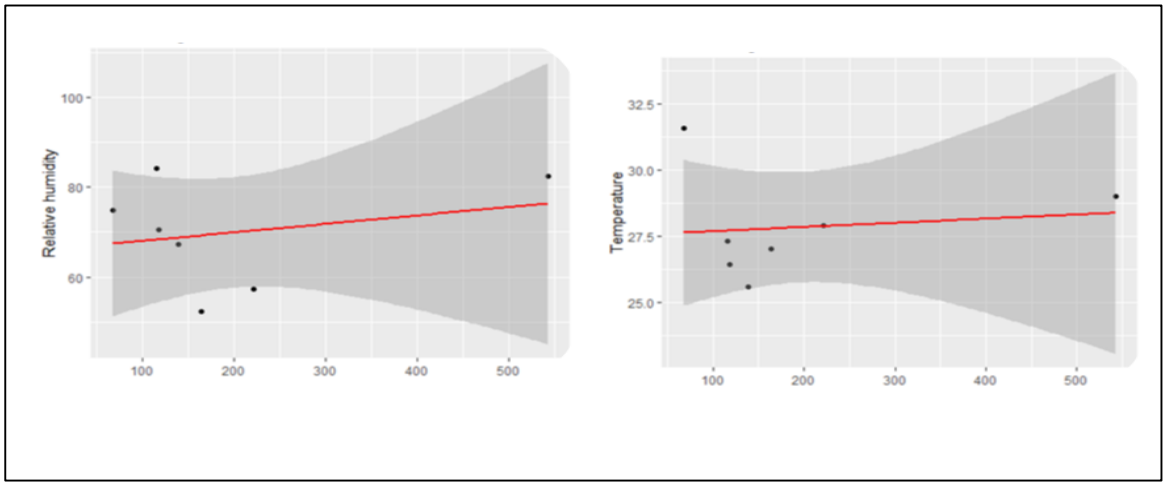

Figure 15: Relation of relative humidity and temperature with distribution of *G. chrysolopha*

4.5 Relation of SHAPU with moth abundance

The yellow hair like setae is found on the abdominal tip of female *G. chrysolopha*, which is released during oviposition for the covering of egg to protect egg from predators and fungi. The setae are thin and thorny structured (Figure 16) which damage the eyes, releasing the fluid from the hollow structure, same as found in ocular sample (Figure18). The comparative study of structures of setae (*G. chrysolopha* and other moths), shows spine like thorny structure on *G. chrysolopha* and smooth structure on other moths

(Figure17). This conclude that the thorny setae of *G. chrysolopha* (Figure 19) damage the morphology of eye and chemicals released from the hollow structure react with the eye component causing the visual impairment.

The numbers of SHAPU patients were higher in the Kaski district, followed by Myagdi and Gorkha districts. The distribution of adult moths was highest in Kaski district, followed by Parbat and Myagdi district. Despite having high distribution of adult in Parbat district the distribution of SHAPU patients were low. The distribution of moth was considerably low in Baglung, Lamjung, and Syangja districts, whereas the numbers of SHAPU patients were also lower in these districts (Figure 20). The number of moth and SHAPU records are highly correlated with each other, increase in moth number increases the number of SHAPU records (Table 4). The setae of moth were found at the eyes of patients from different districts (Figure 18).

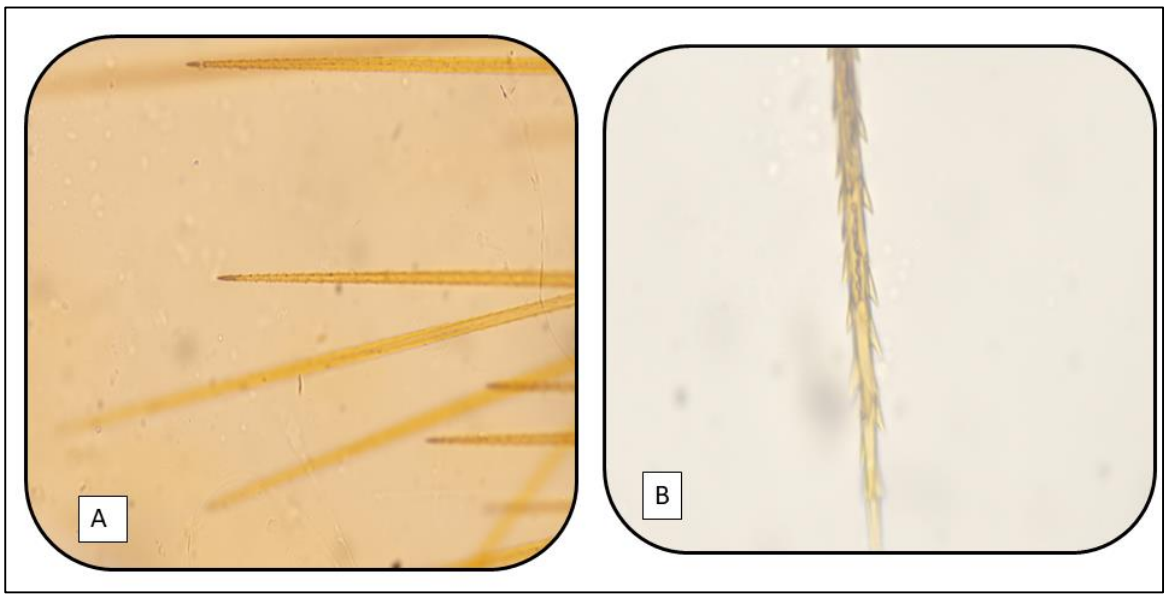

Figure 16: Setae of *G. chrysolopha;* (A) bunch of setae (B) single strand of setae

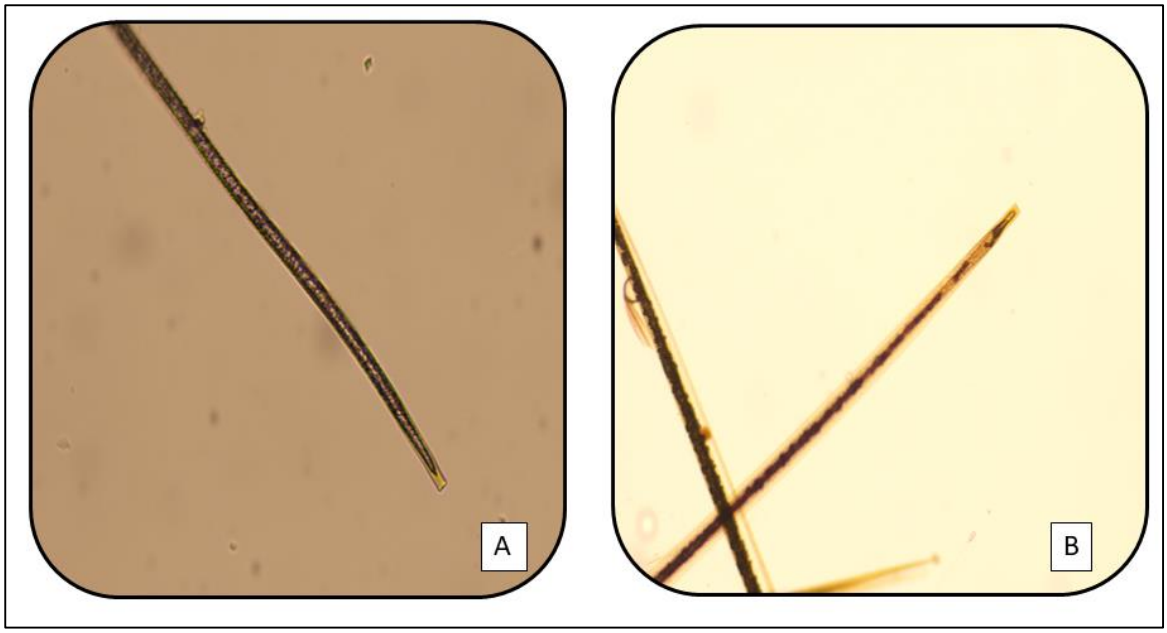

Figure 17: Setae of other moths; (A) setae of *Arctonis* sp. (B) setae of *Thyas* sp.

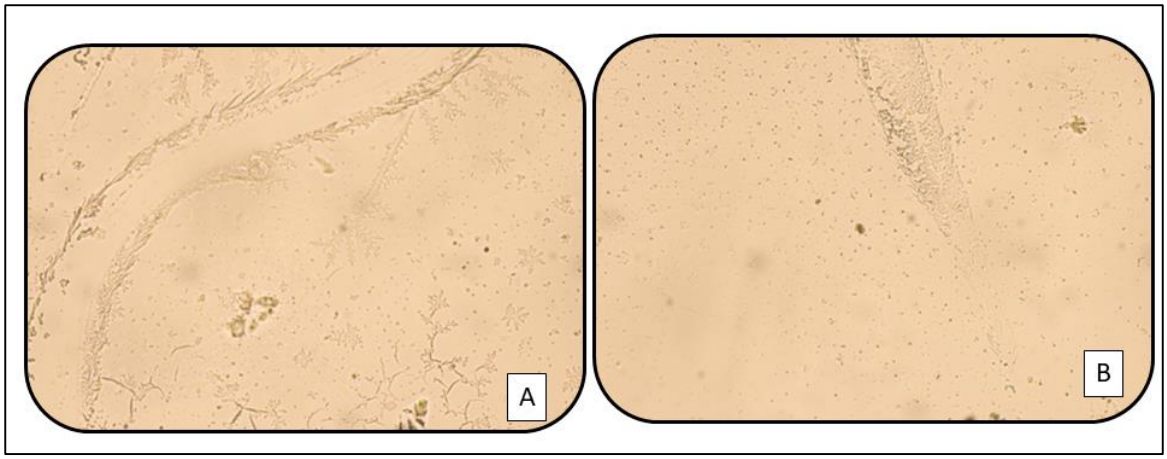

Figure 18: Setae from ocular sample; (A) middle section of setae (B) tip section of setae

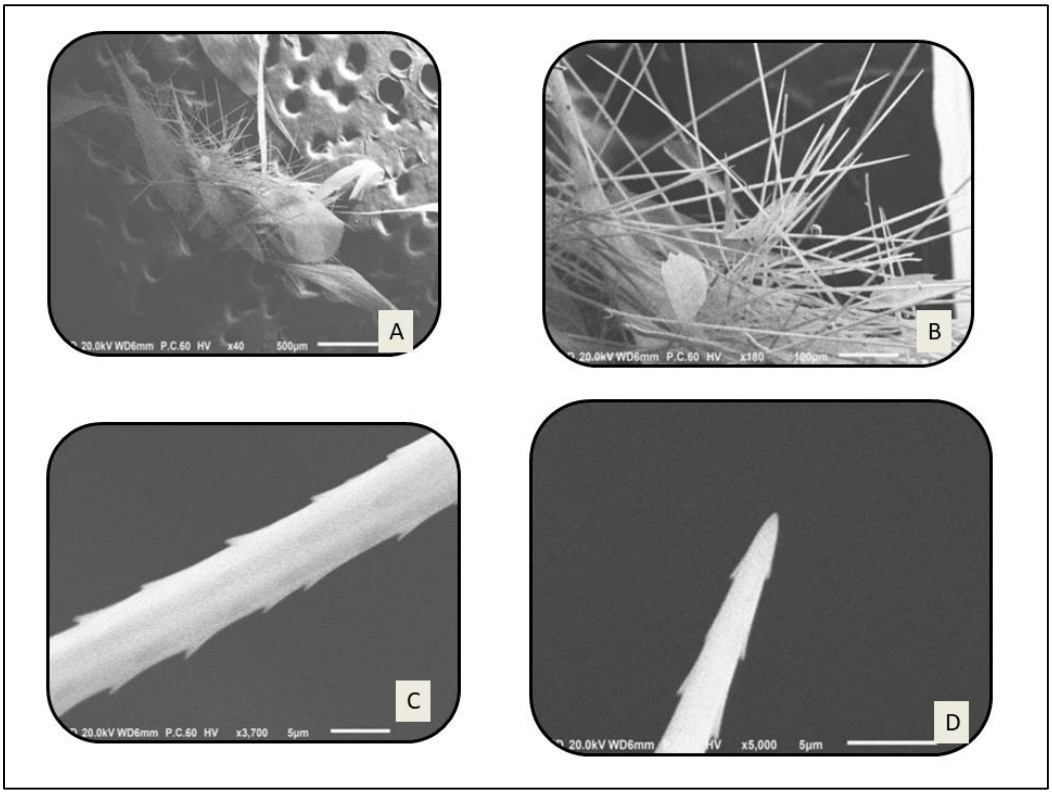

Figure 19: Setae of *G. chrysolopha* captured from electron microscope; (A) Setae mass in 40X (B) Setae mass in 180X(C) Single setae at the trunk part (D) Tip of the setae

Table 4: Correlation between number of moth and SHAPU record

	No. of adult moth(X)	SHAPUrecords
No. of adult moth(X)	1	
SHAPUrecords	0.94	1

Table 5: Number of adults and SHAPU records

Districts	No. of adult moth	SHAPUrecords
Baglung	118	2
Gorkha	164	6
Kaski	543	44
Lamjung	116	1
Myagdi	139	8
Parbat	222	1
Syangja	68	1

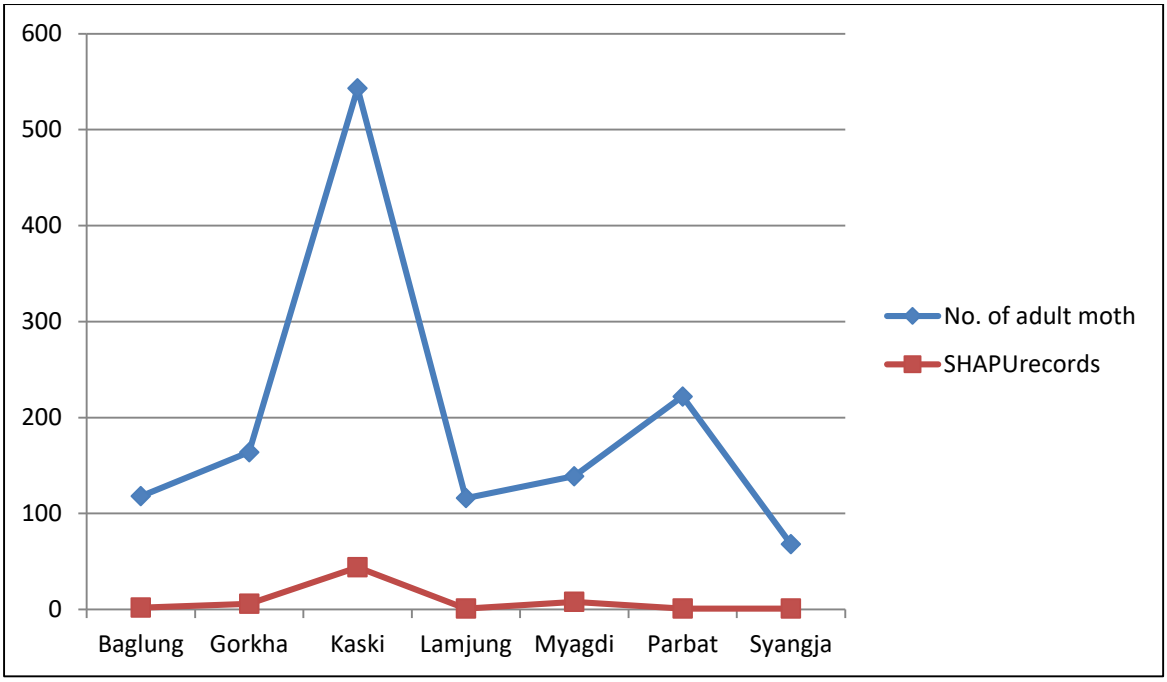

Figure 20: Relation of moth abundance and SHAPU record in seven districts

5. Discussion

This study illustrates about the ecological outlook of the *G. chrysolopha* in Gorkha, Lamjung, Kaski, Baglung, Parbat, Myagdi, and Syangja districts of Gandaki Province, Nepal. The seven districts (Gorkha, Lamjung, Kaski, Baglung, Parbat, Myagdi and Syangja) were selected for the observation and collection of *Gazalina chrysolopha* species at 35 different sites. The sites selection was based on the presence of SHAPU diseases from the ancient record as the moth is considered to be the major suspect. In the Kaski district, adult moths emerge after the monsoon on the odd years, and a few of their un-hatched eggs may hatch under the favorable conditions in the summer of even years, which, after a year-long cycle, result in an outbreak that overlaps newly developed populations of that odd year (Gurung et al., 2021). The *G. chrysolopha* is distributed majorly on mid hilly region and trans-Himalayan terrain (Dewan et al., 2022) The maximum numbers of observation and collection was from outdoor habitat especially from street light. Moths are mostly nocturnal and attracted toward the light sources (Wahlberg et al., 2013).

In this study, we found *G. chrysolopha* oviposit on the dorsal surface of the leaves of the host plant *A. nepalensis* , walls of the houses, poles of the street lights and any objects or plants below the light source with the light brown gelatinous covering. *G. chrysolopha* laid clusters of egg on the surface of the leaves (Rahman & Chaudhry, 1992). The larvae were found on the dorsal surface of leaves and bark of the host plant *A. nepalensis*. The initial instars were abundantly found on *A. nepalensis* whereas third, fourth and fifth instars were found on other plant (*Castanopsis* sp.). Larvae are the major defoliators of the oak trees and *A. nepalensis* in Pakistan and India (Rahman & Chaudhry, 1992; Srivastava, 2003). The pupal stage is found in the soil at the base of host plant inside the cocoon covered with mud. The adult were strictly found in the urban area near the *A. nepalensis* forest. The white moth were observed nearby light sources, in the daytime in resting position on a wall, window glass (Dewan et al., 2022).

The *G. chrysolopha* larva is the nocturnal and shows processionery behaviors, which defoliate the leaves of the host plants. Initial two instars restricted to the *A. nepalensis* whereas later three instars were also observed in the other nearby plants. In 1989, *G. chrysolopha* was observed destroying the oak forest seriously in Bagh Forest Division of Azad, Jammu and Kashmir (Rahman & Chaudhry, 1992). The female adult and larvae

consists setae in the body segments. The comparative structure of *G. chrysolopha* and other moth shows, *G. chrysolopha* consist thorny structure while other moths have smooth setae. The first instar larva is small, light brown with small head capsule and short brown hairs on the body segment. The body length and head capsule increases significantly throughout the instars. The fourth instar has larger hair covering and head capsule. The third instar larva had high mortality rate in natural habitat as well as in the laboratory. The third and fourth instars are active feeder foraging actively on the margin of leaves. Maximum feeding activity of late three instar larvae was recorded at the middle of each stadial period and the larva stopped feeding before molting (Rahman & Chaudhry, 1992). All instar larvae were observed in the bark of tree climbing down in the morning and segregating at the base or at the bark during day time. As larvae are nocturnal they climb up in the night and defoliate the leaves. Larvae damage the leaves of the host plant causing the serious declination of plant species at specific area.

In this study, various bioclimatic factors like temperature, relative humidity does not show statistically significant. The relation between dependent factor (number of moth) and independent factors (temperature and relative humidity) is not strongly significant. The high p-value indicates there is not significant relation between dependent variables and independent variables. The numbers of SHAPU patients are higher at the sites where number of moth abundance is high. The setae presents at the female abdominal tips is considers to be the suspect of SHAPU causing agent in human. The setae were found at the patients eyes, which damage the morphological structure of eyes (Upadhyay et al., 2021).

6. Conclusion and recommendations

6.1 Conclusion

The distribution of *G. chrysolopha* is high where the abundance of *Alnus nepalensis* nearby human settlement. The initial host of the *G. chrysolopha* is *A. nepalensis*. The adult moths emerge in the mid- August to mid- September. Adult moths are attracted toward the light source causing high number of individuals in the street light and household habitat. Adult females lay clusters of egg within 2–4 days of emergence and die. Egg is covered with gelatinous substance produce by female for the protection of eggs from fungi, bacteria and predators. Eggs hatched within 1–2 months. Larvae are voracious feeder within the life stages of the *G. chrysolopha* which defoliates the leaves of host plant *A. nepalensis*. The bioclimatic factors are not significant to the distribution of moth. First instars are small and less voracious whereas second, third, fourth and fifth instars are highly voracious causing severe damage to the host plants. The growth of larva increases subsequently while molting from one instar to another becoming darker and longer in body length & head capsule. The weight of the instars also increases consecutively within the instars. The setae present on abdominal tip of female adult moths are thin and thorny structured which damage morphological structure of the eye. The higher number of moth distribution shows higher number SHAPU patients.

6.2 Recommendations

Based on the results of this study following recommendations have been made:

- The large covering of study area is necessary to identify the ecology of *G. chrysolopha*.
- The intensive study on setae structure is required to analyse the affecting factor for eyes.

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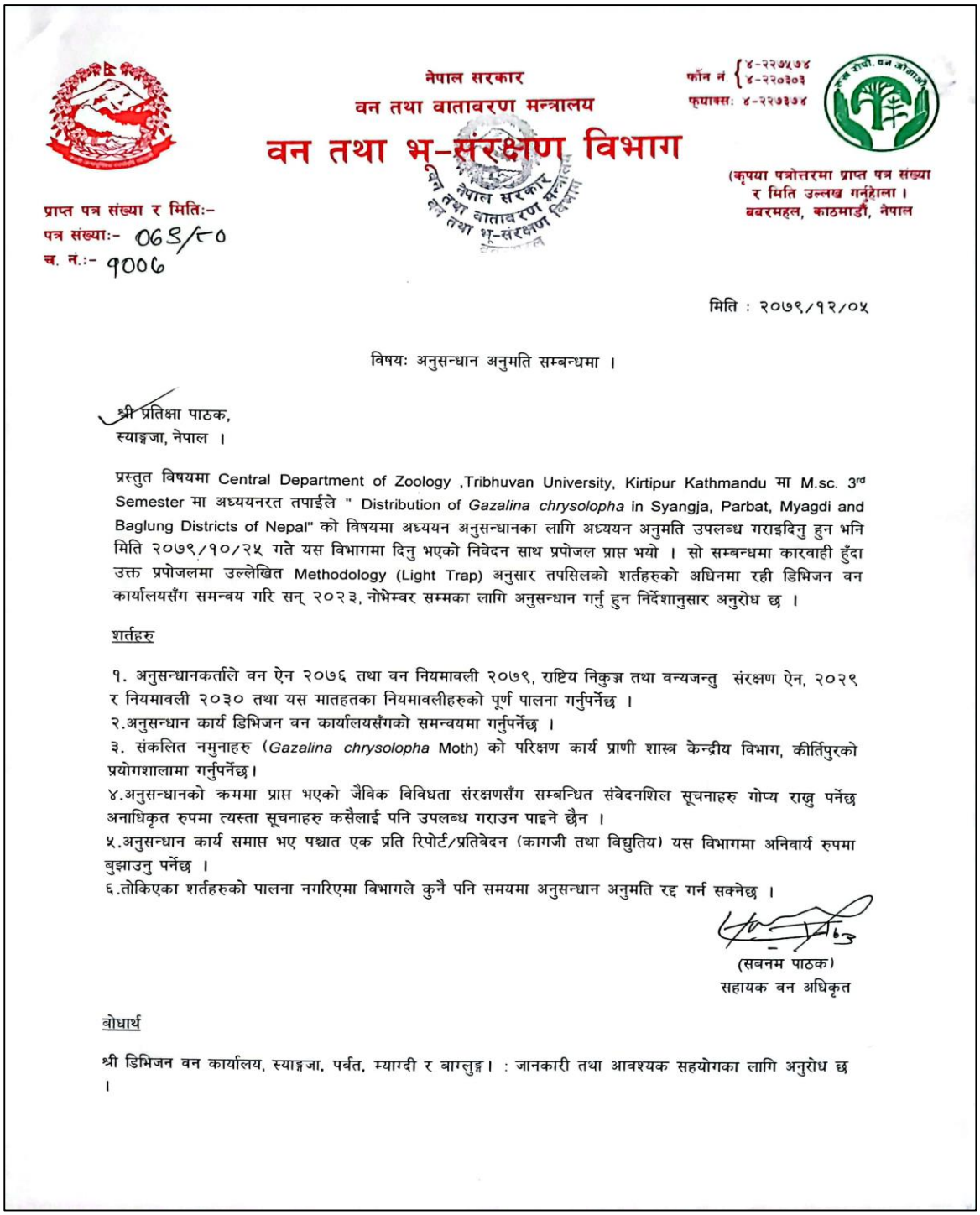
Appendices

Appendix I

Permission letter from Forest and Soil Conservation Department



नेपाल सरकार
वन तथा वातावरण मन्त्रालय
वन तथा भू-संरक्षण विभाग

फोन नं. ४-२२७५७४
४-२२०३०३
फ्याक्स: ४-२२७३७४

(कृपया पत्रोत्तरमा प्राप्त पत्र संख्या र मिति उल्लेख गर्नुहोला ।
बबरमहल, काठमाडौं, नेपाल

प्राप्त पत्र संख्या र मितिः-
पत्र संख्याः- ०६९/८०
च. नं.:- १००६

मिति : २०७९/१२/०५

विषयः अनुसन्धान अनुमति सम्बन्धमा ।

श्री प्रतिक्षा पाठक,
स्याङ्गजा, नेपाल ।

प्रस्तुत विषयमा Central Department of Zoology ,Tribhuvan University, Kirtipur Kathmandu मा M.sc. 3rd Semester मा अध्ययनरत तपाईले " Distribution of *Gazalina chrysolopha* in Syangja, Parbat, Myagdi and Baglung Districts of Nepal" को विषयमा अध्ययन अनुसन्धानका लागि अध्ययन अनुमति उपलब्ध गराइदिनु हुन भनि मिति २०७९/१०/२५ गते यस विभागमा दिनु भएको निवेदन साथ प्रपोजल प्राप्त भयो । सो सम्बन्धमा कारवाही हुँदा उक्त प्रपोजलमा उल्लेखित Methodology (Light Trap) अनुसार तपसिलको शर्तहरुको अधिनमा रही डिभिजन वन कार्यालयसँग समन्वय गरि सन् २०२३, नोभेम्वर सम्मका लागि अनुसन्धान गर्नु हुन निर्देशानुसार अनुरोध छ ।

शर्तहरु

१. अनुसन्धानकर्ताले वन ऐन २०७६ तथा वन नियमावली २०७९, राष्टिय निकुञ्ज तथा वन्यजन्तु संरक्षण ऐन, २०२९ र नियमावली २०३० तथा यस मातहतका नियमावलीहरुको पूर्ण पालना गर्नुपर्नेछ ।
२.अनुसन्धान कार्य डिभिजन वन कार्यालयसँगको समन्वयमा गर्नुपर्नेछ ।
३. संकलित नमुनाहरु (*Gazalina chrysolopha* Moth) को परिक्षण कार्य प्राणी शास्त्र केन्द्रीय विभाग, कीर्तिपुरको प्रयोगशालामा गर्नुपर्नेछ।
४.अनुसन्धानको क्रममा प्राप्त भएको जैविक विविधता संरक्षणसँग सम्बन्धित संवेदनशिल सूचनाहरु गोप्य राख्नु पर्नेछ अनाधिकृत रुपमा त्यस्ता सूचनाहरु कसैलाई पनि उपलब्ध गराउन पाइने छैन ।
५.अनुसन्धान कार्य समाप्त भए पश्चात एक प्रति रिपोर्ट/प्रतिवेदन (कागजी तथा विद्युतिय) यस विभागमा अनिवार्य रुपमा बुझाउनु पर्नेछ ।
६.तोकिएका शर्तहरुको पालना नगरिएमा विभागले कुनै पनि समयमा अनुसन्धान अनुमति रद्द गर्न सक्नेछ ।

(सबनम पाठक)
सहायक वन अधिकृत

बोधार्थ

श्री डिभिजन वन कार्यालय, स्याङ्गजा, पर्वत, म्याग्दी र बाग्लुङ्ग। : जानकारी तथा आवश्यक सहयोगका लागि अनुरोध छ ।

Annex 1

CDZ Reg. No.	Location
CDZMTU-LEP-01001	Majhuwa khola
CDZMTU-LEP-01002	Majhuwa khola
CDZMTU-LEP-01003	Majhuwa khola
CDZMTU-LEP-01004	Pokhara-17
CDZMTU-LEP-01005	Birauta chowk
CDZMTU-LEP-01006	Seti dam
CDZMTU-LEP-01007	Godgode
CDZMTU-LEP-01008	Godgode
CDZMTU-LEP-01009	Syangja district hospital

The detail of the specimens deposited in the museum of Central Department of Zoology

Annex 2

GPS coordinates of the study area

District	Sites	Latitude(N)	Longitude(E)
Baglung	Baglung-6 (new buspark)	28.28044	83.59766
Baglung	Kathekhola-1	28.26539	83.5436
Baglung	Kudule	28.29	83.5935
Baglung	Upallachaur	28.2787	83.6009
Baglung	Dhorpatan-1	28.3298	83.161
Myagdi	Beni-8	28.3507	83.564
Myagdi	Beni-9 (kaulegauda)	28.3499	83.559
Myagdi	Beni-2 (Khapra)	28.3395	83.569
Myagdi	Beni-6 (Arthunge)	28.345	83.553
Myagdi	Beni-4	28.34	83.55
Parbat	Kusma- 5	28.224	83.674
Parbat	Kusma-11	28.2205	83.7105
Parbat	Kusma-4	28.231	83.672
Parbat	Kusma-10	28.212	83.706
Parbat	Kusma- 6	28.218	83.681
Gorkha	Gorkha-6 (Haramtar)	27.993914	84.627054
Gorkha	Gorkha-8 (Kholkhole)	27.9876	84.6978
Gorkha	Shahid lakhan ga. Pa.-7	27.9395	84.6872
Gorkha	Gorkha-7	27.999	84.6152
Gorkha	Gorkha-6(Darbar)	28.0038	84.6265

Kaski	Pokhara- 17, Birauta powerhouse	28.18164	83.97073
Kaski	Pokhara-9, Prithivi chowk	28.20937	83.98566
Kaski	Pokhara -7, Mustang chowk	28.19831	83.9759
Kaski	Pokhara -17 Birauta	28.1924	83.97174
Kaski	Pokhara ward-13 seti dam side	28.248894	83.97337
Lamjung	Besisahar-3, Majhuwakhola	28.2243	84.38015
Lamjung	Besisahar-7, Lamjung	28.2369	84.3728
Lamjung	Besisahar, Lamjung	28.2278	84.3773
Lamjung	Besisahar-8, Lamjung hospital	28.2297	84.38004
Lamjung	Besisahar-8,near marsyangdi river	28.23306	84.3823
Syangja	Putalibazar1, Sahid chowk	28.10558	83.87364
Syangja	Putalibazar -3, godgode	28.1111	83.8785
Syangja	Waling -13, Udiyachour	27.9756	83.7095
Syangja	Galyang -3, Tallogalyang	27.9471	83.6768
Syangja	Putalibazar -3, Syangja hospital	28.10558	83.8742

Appendix II

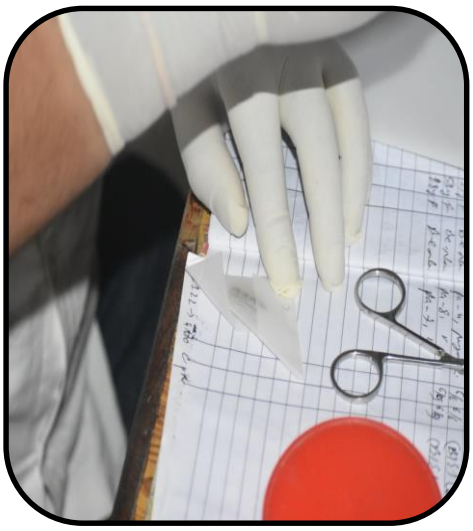
Light trap set

Direct hand collection

Male *G. chrysolopha*

Female *G. chrysolopha*

Appendix III



Collection of specimen

Collection of physical parameters

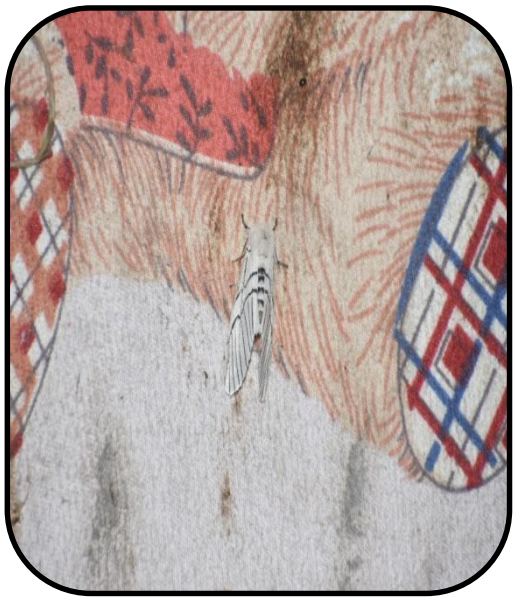
Host plant *A. nepalensis*

Preservation of specimen in envelope

Appendix IV

Moth on the outdoor light source

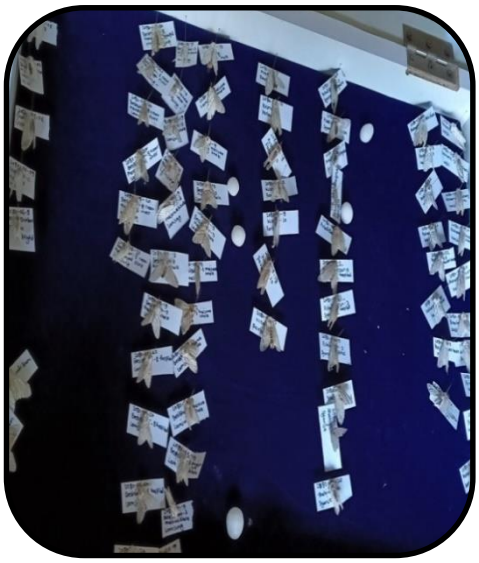
Moth on the street light pole



Moth on the cloths

Natural predator of *G. chrysolopha*

Appendix V



Preservation of collected specimens

Egg on the dorsal side of host plant leaf

Cluster of larva on the bark of host plant

Shedding of larva on trunk of the host plant