STATUS OF *Tuta absoluta* LARVA IN TOMATO GROWING SITES OF KATHMANDU VALLEY

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A thesis submitted in partial fulfilment of the requirements for the award of the degree of Master of Science in Zoology with special paper Entomology.

Submitted to

Central Department of Zoology
Institute of Science and technology
Tribhuvan University
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September 2023

DECLARATION

I hereby declare that the work presented in this thesis entitled "**Status of *Tuta absoluta* in Tomato Growing Sites of Kathmandu Valley**" 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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LETTER OF APPROVAL

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Ms. Sharmila Adhikari entitled **"Status of *Tuta Absoluta* in Tomato Growing Sites of Kathmandu Valley"** has been accepted as a partial fulfillment for the requirement of Master's Degree of Science in Zoology with special paper Entomology.

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LIST OF ABBREVIATIONS

CDZ	Central Department of Zoology
GLM	Generalized Linear Model
GPS	Global Positioning System
IPM	Integrated Pest Management
Asl	Above Sea Level
p value	Probability Value
T. absoluta	*Tuta absoluta*

ABSTRACT

This research was conducted to explore the status of *Tuta absoluta* larvae in Kathmandu Valley, analyze its seasonal variation, explore the relation of larvae with physical parameters (temperature and humidity). Three study sites Dahachowk (site 1), Gundu (site 2) and Naikap (site 3) were selected. *T. absoluta* larvae were collected from these study sites from April 2019 to January 2020 with monthly visit to each sites using quadrat method (10×10 m^2). There were varying levels of abundance of *Tuta absoluta* among the three sites. In terms of abundance, Dahachowk area (site 1) showed highest abundance of larvae (12.7 ± 4.9) whereas least abundance at Naikap site (site3) (11.4 ± 6.5). During autumn, *T. absoluta* populations were the highest and least during winter. Different sites have different interactions with temperature and humidity regarding *T. absoluta* larvae populations. Humidity and temperature showed statistical significance on dependent variable i.e., larval abundance on all the sites. To mitigate the impact of *T. absoluta* on the Kathmandu Valley's agricultural community, tailored integrated pest management practices must be implemented according to site-specific environmental conditions. As a result of identifying the distribution and abundance of this invasive pest, targeted control measures can be developed and crop losses can be reduced.

सार

यो अनुसन्धान काठमाडौँ उपत्यकामा *Tuta absoluta* लार्भाको अवस्था पत्ता लगाउन, यसको मौसमी भिन्नताको विश्लेषण गर्न, भौतिक मापदण्डहरूसँग लार्भाको सम्बन्ध पत्ता लगाउन गरिएको थियो। (temperature and humidity). तीन अध्ययन स्थल दहचौक (स्थल 1) गुन्डु (स्थल 2) र नाइकाप (स्थल 3) चयन गरियो। *T. absoluta* लार्भा क्वाड्र्याट विधि (10 × 10 m^2) प्रयोग गरेर प्रत्येक साइटहरूमा मासिक भ्रमणको साथ अप्रिल 2019 देखि जनवरी 2020 सम्म यी अध्ययन साइटहरूबाट सङ्कलन गरिएको थियो। तिनवटा स्थलहरूमा *T.absoluta* प्रशस्तताको विभिन्न स्तरहरू थिए। प्रशस्तताको सन्दर्भमा, दहचौक क्षेत्र (साइट 1) ले लार्भा (12.7 ± 4.9) को उच्चतम प्रशस्तता देखायो जबकि नाइकाप साइट (साइट 3) मा न्यूनतम प्रशस्तता (11.4 ± 6.5) शरद ऋतुमा, टि. एब्सोलुटा जनसङ्ख्या सबैभन्दा बढी र जाडोमा सबैभन्दा कम थियो। विभिन्न साइटहरूमा *T. absoluta* लार्भा जनसङ्ख्याको सन्दर्भमा तापमान र आर्द्रतासँग फरक-फरक अन्तरक्रिया हुन्छ। आर्द्रता र तापमानले निर्भर चर i.e., सबै साइटहरूमा लार्भा प्रशस्ततामा सांख्यिकीय महत्त्व देखायो। काठमाडौँ उपत्यकाको कृषि समुदायमा *T. absoluta* प्रभाव कम गर्न, साइट-विशिष्ट वातावरणीय अवस्था अनुसार अनुकूलित एकीकृत कीट व्यवस्थापन अभ्यासहरू कार्यान्वयन गर्नुपर्छ। यस आक्रामक कीटको वितरण र प्रशस्तता पहिचान गर्ने परिणामको रूपमा, लक्षित नियन्त्रण उपायहरू विकास गर्न सकिन्छ र बालीको क्षति कम गर्न सकिन्छ।

1. INTRODUCTION

1.1 Background

Tuta absoluta is one of the serious South American tomato insect pests (Cuthbertson et al., 2013; Oztemiz, 2013). The tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is one of the most devastating invasive pests affecting tomato crops (*Solanum lycopersicum*) in many parts of the world. It is native to South America and its geographic distribution is rapidly expanding. Initially this pest was detected in Europe in eastern Spain in 2006 (Urbaneja et al. 2007) and continued to invade different European countries and countries facing Mediterranean Sea causing severe damage to open field and greenhouse tomatoes (Deseneux et al. 2010). It is observed on tomatoes growing in an altitude ranging less than 1000m above the sea level (Tonang et al. 2015). This pest can easily survive in severe environmental conditions such as cold and hot tropical regions (Cuthbertson et al. 2013). The pest can survive on high temperature of $49^{O}C$ in summer (Tonang et al. 2015) and low temperature below $5^{O}C$ (Vandamme et al. 2015). It was first recorded in 1917 and as tomato pests in 1960s in Peru (Seplyarsky et al. 2010; Guedes and Picano, 2012). Later its invasion was seen in rest part of Europe like France, Italy, Greece, Malta and Bulgaria (Harizanova et al. 2009; Roditakis et al. 2010; Braham et al. 2012). Its identification from Algeria and Morocco in 2008, Libya in 2009 (Harbi et al. 2012) depicts its growing distribution pattern to African region. Its occurrence in dry and hot regions in Africa suggests that leaf miner is strongly heat tolerant and can survive as long as host plants are available. In contrast, cold winters, lack of soil moisture or drought stress may be the key factors that lower the risk of permanent establishment in Northern Europe, most of Russia and Canada (Tonnang et al. 2015). Moreover, tomato greenhouses and plant nurseries may help to build up the population and survive unfavorable climatic conditions. These sites are hot spots, perhaps acting as bridgeheads for further invasion (VanDamme et al. 2014).

T. absoluta undergoes complete metamorphosis and reproduces rapidly. There are four stages to its life cycle: egg, larvae, pupae, and adults, which usually take around 24 days to complete at 27°C (Gebremariam 2015, Rasheed et al. 2018). The life cycle of an insect begins when its adult form lays its eggs on the leaves, buds, stems, and unripe fruits of plants (Tropea Garzia et al. 2012). As the eggs mature, they begin as oval shaped and creamy white, eventually turning yellow and eventually turning black (Rasheed et al. 2018). There is a short period before egg-laying known as the pre-oviposition period that lasts between 2.3 and 4.6 days, depending on the temperature (Tropea Garzia et al. 2012, Rasheed et al. 2018). *T. absoluta* is a small moth with a body length of 5-6 mm and a wing span of 8-10 mm (Bajracharya et al. 2016).

It was first reported in India in 2014 (Shashank et al. 2015) and Bangladesh in 2016 (Hossain et al. 2016). The insect pest *T. absoluta* was first recorded in Nepal in May 2016, specifically in Kathmandu (Bajracharya et al. 2016). Thereafter, it spread rapidly to tomato fields around Kathmandu valley (Bajracharya et al. 2016). There have been recent reports in 23 out of 77 districts of Nepal in tomato-growing areas. Most of the infestations have been observed on the plains and the mid-hills of the country (Bajracharya & Bhat 2018). Consequently, *T. absoluta* has spread to a number of tomatoes growing regions in Nepal and has had a significant impact on them.

The Kathmandu Valley of Nepal has experienced a significant problem with *T. absoluta* infestation (Bajracharya et al. 2016). In the region, tomato production is threatened by the presence of this invasive pest. The effects of pests on tomato crops include yield loss, reduced quality, and increased production costs. *T. absoluta* larvae feed on tomato leaves, stems, and fruits, creating tunnels and galleries within the tissues (Bajracharya et al. 2016). As a result of this feeding activity, the plants become weaker, slow their growth, and in severe infestations, they can even die (Bajracharya & Bhat 2018). Due to the pest's damage, fruits become less marketable and more susceptible to secondary infections (Bajracharya & Bhat 2018, Bacci et al. 2019).

The seasonal dynamics of insect pest populations are influenced by a variety of factors, such as resource availability, climatic conditions, natural enemies, and competition with other species (Sylla et al. 2018). There is a direct correlation between climatic factors such as temperature, humidity, wind, and rainfall and critical activities such as oviposition, feeding, growth, development, reproduction, and migration of these pest (Bacci et al. 2019, Bacci et al. 2021). It is believed that *T. absoluta* is influenced by temperature in terms of development and activity (Krechemer & Foerster 2019). The development of pests depends on warm temperatures, usually ranging from 25°C to 30°C (Asgari & Fathipour 2019). Pests grow and reproduce faster at higher temperatures, which accelerates their life cycle (Nayana et al. 2018) . The population of *T. absoluta* is at risk from extreme hot or cold temperatures, both of which can affect the organism (Asgari & Fathipour 2019). Additionally, moth activity and mobility are affected by the temperature, which affects their ability to disperse and find suitable hosts. It is also important to consider the effects of humidity on *T. absoluta* populations. A high humidity level provides ideal conditions for pest reproduction and survival (Tadele & Emana 2017). The presence of moisture facilitates the hatching of eggs and the development of larvae. It is also possible for high humidity to promote fungal pathogen growth, which may have an indirect effect on *T. absoluta* populations (Brévault et al. 2014). Furthermore, excessively low humidity can negatively affect the survival and development of pests (Guimapi et al. 2016) . Besides temperature and humidity, host plant availability, crop management practices, and natural enemies also influence *T. absoluta* populations (Guimapi et al. 2016) .

1.2 Objective of research

1.2.1 General objective

- To find the status of *T. absoluta* larva in tomato growing sites of Kathmandu Valley.

1.2.2 Specific objectives

- To assess the abundance of *T. absoluta* larva in difference sites of Kathmandu Valley.
- To seasonal variation of *T. absoluta* larva in different sites of Kathmandu Valley.
- To evaluate the relation of *T. absoluta* larva with physical parameters (temperature and humidity).

1.3 Significance of the study

Kathmandu Valley is known for its diverse agricultural practices and high vegetable production. It contributes to the local economy and meets the food needs of the region since tomato cultivation is one of the most important agricultural activities in the region. However, tomato crops in the valley are threatened by *T. absoluta* infestation, threatening sustainable production. *T. absoluta* infestation has been reported in Nepal since 2016, and its impact on tomato crops has concerned farmers, researchers, and policymakers. Nevertheless, there is limited knowledge regarding *T. absoluta*'s abundance, distribution, and factors affecting its infestations in tomato crops within the Kathmandu Valley. In order to develop effective pest management strategies, it is crucial to understand *T. absoluta* infestation patterns, factors that influence pest spread, and seasonal variation. The presence and distribution of a pest can help farmers implement targeted control measures, reduce crop losses, and improve production practices. In addition, it may be beneficial to explore the relationship between *T. absoluta* and physical parameters, such as temperature and humidity, in order to develop preventative measures and optimize crop management techniques.

2. LITERATURE REVIEW

2. 1 Overview of *Tuta absoluta* and its impact on Tomato crops

Tuta absoluta is commonly known as the South American tomato moth or tomato leaf miner. It is an extremely destructive pest that poses a significant threat to tomato crops worldwide (Shashank et al. 2015, Poudel & Kafle 2021). This invasive species originated in South America, primarily in Peru (Gebremariam 2015). It spread rapidly from South America to Europe, North Africa, and the middle East (Tropea Garzia et al. 2012). In South Asia, it was observed in India in 2014 (Shashank et al. 2015), Bangladesh in 2016 (Hossain et al. 2016). It has also been reported to occur in Qatar 2010, Oman 2011, Keyna 2013, and Afghanistan 2015 (Hossain et al. 2016). Similarly, it was observed first time in China 2017 (Zhang et al. 2020). In Nepal, It was first observed in Kathmandu valley in 2016 and eventually spread to surrounding tomato-growing regions (Bajracharya et al. 2016). It was noted in 2016 that infestations were beginning in several districts, including Kathmandu, Lalitpur, Bhaktapur, Kavre, and Dhading (Bajracharya et al. 2016). In 2018, 33 districts in Nepal where tomatoes are cultivated had been affected by the pest (Bajracharya & Bhat 2018). As a result, food security and substantial economic losses have been suffered in various regions of the country (Poudel & Kafle 2021). *Tuta absoluta* has a relatively short life cycle, surviving 24 to 76 days per generation under optimal conditions (Gebremariam 2015, Bajracharya & Bhat 2018). It goes through four main stages of development: egg, larva, pupa, and adult (Rasheed et al. 2018, Poudel & Kafle 2021) and is completed within 24 days at 27^{0}C (Gebremariam 2015). In the larval stage, the larvae feed on leaves, stems, and fruits, causing extensive damage to the plant (Tropea Garzia et al. 2012). Tuta absolute has a very significant effect on both outdoor and greenhouse tomato cultivation (Tropea Garzia et al. 2012). It has caused severe losses of 80-100% in tomato crops, both in enclosed and open-field areas (Poudel & Kafle 2021). As a result of the pest's direct feeding and wounds it causes on plants, secondary infections can occur, which impacts both yield and quality (Tropea Garzia et al. 2012). The larvae are responsible for feeding damage throughout the crop growth cycle at all stages. A tomato plant's buds, flowers, new fruits, leaves, and stems are infested by the larvae (Bajracharya et al. 2016). A larva that feeds on leaves forms irregular mines that can turn necrotic, while a larva that feeds on stems forms extensive galleries that alter the growth of the plant (Gebremariam 2015, Bajracharya et al. 2016). Likewise, larvae attack fruits, forming galleries that provide entry points for secondary pathogens, ultimately resulting in fruit rot (Gebremariam 2015, Bajracharya et al. 2016). There are a variety of control methods available, including chemical insecticides, biological agents, mass traps that use pheromones, and botanical extracts (Illakwahhi & Srivastava 2017, Joshi et al. 2018). *Tuta absoluta*, originally from South America, has become an invasive pest of tomatoes, causing significant damage. It quickly spread to various European countries and became a major agricultural pest. In order to develop effective plant protection strategies, it is important to understand the plant's key biological characteristics. It is a multivoltine species characterized by consistent behavior patterns. Environment factors, specifically temperature, influence its life cycle. A larvae of this pest feeds and develops inside tomato leaves, stems, and fruits during their entire growth cycle. In the Mediterranean region, adults are active during twilight hours. *T. absoluta* damage directly impacts plants' photosynthetic capacity and reduces production levels in both protected and open-field tomato crops. Additionally, secondary infections can occur as pathogens develop on infested plant and fruit tissues, leading to further damage (Tropea Garzia et al. 2012). Adhikari et al. (2019) studied monitoring and management of tomato leaf miner in Kavrepalanchowk, Nepal from December 2016 to October 2017. It was reported that the incidence of *Tuta absoluta* increased during the transplanting season, from June to July, and then decreased gradually thereafter. Various management techniques have been used, including cultural, physical, mechanical, quarantine, chemical attractants, and botanicals. Farmers also adopted other chemical practices. It was found to be infested in all seasons, although the level of infestation differed based on management practices.

2.2 Relationship between *Tuta absoluta* and environmental variable

Population dynamics of *T. absoluta* are strongly influenced by environmental, biological, and agricultural factors. This pest's population fluctuation is affected directly or indirectly by many factors (biotic and climatic), such as plant age, mirid bugs, air temperature, relative humidity, and soil temperature (Rizk et al. 2015). Temperature significantly affects insect’s population. Warmer temperatures are beneficial to *Tuta absoluta* populations (Nayana et al. 2018, Asgari & Fathipour 2019).

Pests' life cycle can be accelerated by higher temperatures, resulting in more generations and a larger population (Martins et al. 2016). *T. absoluta* emerged at a temperature of 18.0°C on average, with a maximum temperature of 27°C and a minimum temperature of 10.4°C. It was observed that *T. absoluta's* peak population occurred in April when the average temperature was 26.1°C. With higher rainfall levels, *T. absoluta* was found to be more evident, as determined by a linear relationship between rainfall and its presence (Soti et al. 2020). Besides being ecologically beneficial to *T. absoluta*, humid environments are conducive to its survival and reproduction (Tadele & Emana 2017) . Population density can increase with higher humidity levels. The monsoon season or areas with high humidity are particularly conducive to pest infestations (Bacci et al. 2019, Saad et al. 2020). Abdurrahman & MUTLU (2019) observed that *Tuta absoluta's* population density peaked in September, followed by a decline. It was found that ferolite traps were particularly effective at capturing adults, capturing an average of 278 a week, which highlights their potential as a pest control tool in tomato production areas. Similarly, Asgari & Fathipour (2019) investigated seasonal activity and population fluctuations of the tomato leaf miner (*T. absoluta*) in Tehran province during 2015-2016. There was an approximately one-month difference between 2015 and 2016 in the peak number of moths trapped in the traps. The larval density reached its highest point in 2015 with 2.34 larvae per plant and 10.8 larvae in 2016. Temperatures between 25 and 30°C were preferred by the tomato leaf miner, and the interval between successive peaks decreased with temperature. According to larval growth rates, the pest completed three generations within a single growing season in both years. The trap data indicates that there are 11 generations within an annual cycle, with the 11th generation overwintering during the winter months. As a result of temperature and host availability, multiple generations of the pest can occur within one growing season. *T. absoluta* life cycles over time were directly affected by insecticide application, host plant availability, and climatic conditions, resulting in shifts in population peaks. It was not possible to reduce pest densities below economic injury levels during population growth periods with insecticides, which reduced larval damage. During August and January, the highest densities of mined and damaged fruits were observed, while between September and January, they were observed in crops treated with insecticides. The highest pest densities were also associated with periods where air temperatures were rising and rainfall was low. In order to mitigate damage and optimize crop yields, tomato phenology, climatic conditions, and pest control strategies need to be considered when making management decisions regarding its management (Bacci et al. 2021). In India found that *Tuta absoluta* infestation levels were positively correlated with maximum temperatures. Based on their findings, early pest management strategies seem to be important to mitigate negative effects on tomato crops throughout the season. It is directly linked to the presence of suitable host plants, such as tomato crops, that the populations of *Tuta absoluta* increase (Guimapi et al. 2016). Abundant host crops provide a refuge for a greater number of pests (Guimapi et al. 2016) . A number of other factors contribute to the control of *Tuta absoluta* populations, including predators, parasitoids, pathogens, and natural enemies (Bacci et al. 2019). Besides tomatoes, Tuta absolute feeds on other solanaceous plants. A pest's distribution and survival may be influenced by the availability of alternative host plants (Mohamed et al. 2015). A variety of agricultural practices can affect pest populations indirectly by altering the health and vigor of the plants (Chhetri 2018). These practices include irrigation, fertilization, and pruning (Chhetri 2018). It´s populations may develop pesticide resistance from misuse or overuse of chemical pesticides. Control measures can be less effective when pesticide resistance exists (Braham et al. 2012, Moussa et al. 2013) . There is a wide range of susceptibility to *T. absoluta* infestations among different tomato varieties. Pest distribution can be affected by cultivars that exhibit higher resistance (Never et al. 2017). It can spread to new areas through trade or transportation of infested plant material, tomato fruits from infested areas, and packaging equipment (Gebremariam 2015). It was found that farmers' potential adoption was influenced primarily by sex, distance from inputs, distance from the nearest agricultural extension office, training, knowledge level, attitude level, and practice level (Chepchirchir et al. 2021, Chepchirchir et al. 2021). The tomato leaf miner, *T. absoluta*, has recently become a major pest in India, affecting both open field and protected tomato crops. T. absolute infestation levels were initially low during the first crop phenological cycle, but gradually increased as crops aged in both polyhouses and fields. It was found that the incidence of *T. absoluta* was positively correlated with the maximum temperature during both the Kharif and Rabi seasons. Consequently, to reduce *T. absoluta* populations later in the phenological cycle, pest management strategies should be implemented during the early growth period. The negative impacts of *T. absoluta* infestations on tomato crops can be mitigated by early intervention (Nayana et al. 2018) .

3. MATERIALS AND METHODS

3.1 Study Area

The Kathmandu Valley located in central Nepal; a fertile plain is considered one of the most significant agricultural regions in the country. The valley is surrounded by hills and mountains and irrigated by several rivers, making it an ideal location for crop production. The study area includes Dahachowk (sites 1), Gundu (site 2) and Naikap (site 3) areas of Kathmandu Valley. Dahachowk and Naikap lies in Chandragiri Municipality of Kathmandu district and Gundu lies in Suryabinayak Municipality of Bhaktapur district (Fig.1). Chandragiri Municipality is bordered by Kirtipur Municipality in east, Dhunibeshi Municipality in west, Nagarjun Municipality in north and Daksinkali Municipality in south. It extends between 27°43' 36" N to 85°16' 39" E in the east. The altitude variation of these areas lies between 1310 and 2551 asl. Gundu (sites 2) lies in the southern region of Bhaktapur, extending between 27°38' 25" N to 85° 25' 6" E with an altitude range between 1372 to 2025m above sea level. The vegetation of study area is of subtropical type and most of the area is under cultivation. The study area mainly comprises of crop fields, agricultural lands, vegetable fields and urban areas.

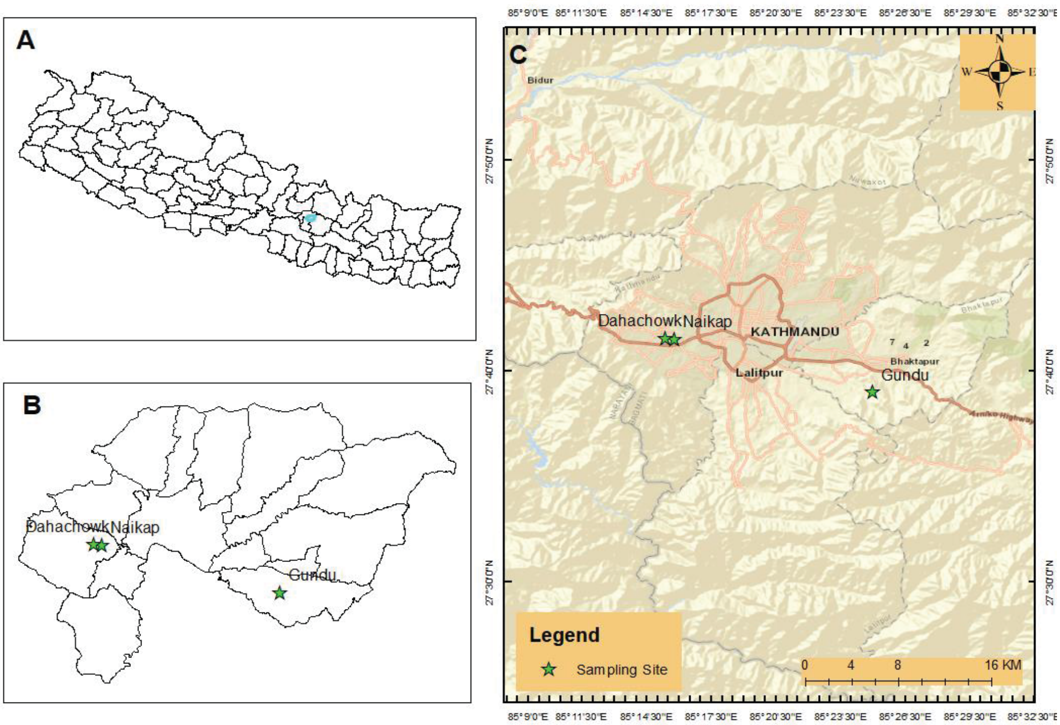

Figure 1. Map of Nepal showing Kathmandu Valley (B) Map of Kathmandu valley showing Study area (C) Map of study area showing sampling points.

The study area lies in a subtropical climate zone (1000-2000 meters) and deciduous monsoon forest zone (1200-2100 meters). The highest temperature is recorded during May and June whereas the lowest temperature is recorded in December and January.

3.2 Materials

Materials used for the study include:

- Small vials
- Hair brush
- Polythene bags
- Hygrometer (for temperature and humidity data)
- Forceps
- Ethanol (70%)
- Gloves
- GPS
- Datasheet
- Quadrat (10×10m^2)
- Olympus stereomicroscope
- Compound microscope

3.3 Sampling method

Site selection and Field design

For this research, 3 study sites were chosen. The study sites were selected based on visual observation and oral communication with farmers. The study was conducted on tomatoes grown in greenhouse tunnels. These 3 study areas are considered 3 sampling sites. Each sampling site was visited once every month throughout the study period, which was from April 2019 to January 2020. Quadrat methods are used for sample collection.

Sampling and Data collection

A quadrat of (10×10 m^2) was laid down thrice at each sampling site. The larvae of *T. absoluta* were observed thoroughly in each quadrate using roving/fixed plot survey methods. The samples were collected mainly from leaves region of plants in vials with 70% ethanol as a preservative and with the date, season, site and condition of the host plants. Hygrometer recorded temperature and humidity data. A separate data sheet was used to record all of these details. Digital pictures of *T. absoluta* and host plants were also taken.

3.4 Identification and Deposition

Samples collected were transported to CDZ Entomology Laboratory. Different magnifications were used to observe them under a stereomicroscope. Larva of *T. absoluta* was identified based on its morphological characteristics with the help of Dichotomous Identification keys (Passoa and Young, 2007).

3.5 Data analysis

Descriptive statistics, such as means and medians, standard deviations and bar diagrams using MS Excel 2013. Generalized linear model was used to analyze the relation between *T. absoluta* and physical parameter (temperature and humidity) variables by R-software (r-3.4.1).

Table 1: Diagnostic Characteristics of *Tuta absoluta* larvae in the study area

S. N	Diagnostic characteristics of *T. absoluta*
1	A trisetose prespiracular group.,SV group on A1 bisetose., A1-8 with L1 and L2 closely spaced., A9 with at least SD1 and D1 in a vertical line and D2 in its own pinnaculum.
2	Prothoracic shield with a dark band on posterior margin of head
3	Profile of dorsum of A7 and A8 has micro spines under higher magnification., abdomen never has irregular bands or patches of pigmentation in either live or preserved specimens

A

B

C

D

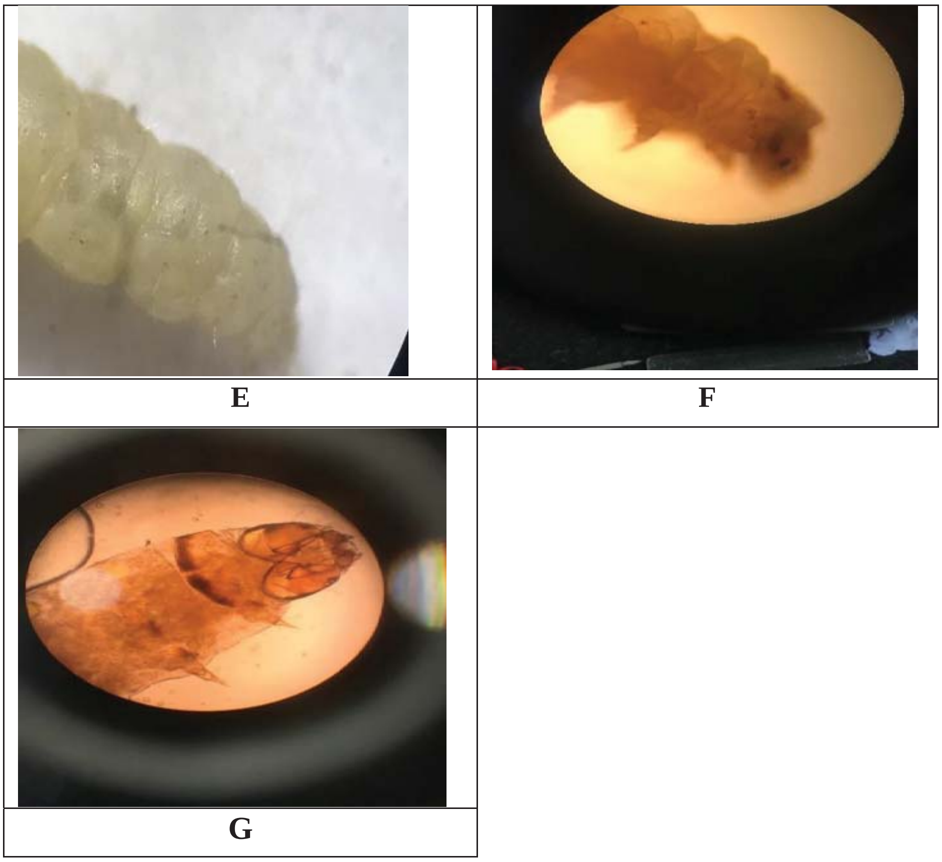

Fig 2: Different morphological features of *T. absoluta* larva A-B: Immature stages of larvae, C-D: Head region with prothoracic shield in different magnification, E: Abdominal segment of larvae, F: Thoracic segment with setae, G: Head region of larvae in permanent slide

4. RESULTS

4.1 Abundance of *Tuta absoluta* larva

A total of 342 *Tuta absoluta* larvae were collected at site 1 with a mean of 12.7 ± 4.9. Likewise, at site 2, there were 310 larvae, with a mean count of 11.5 ± 6.9. Additionally, at site 3, a total of 308 larvae were observed, with a mean count of 11.4 ± 6.5. A statistically higher level was found at site1, followed by site 2 and the least at site 3 (Fig. 2).

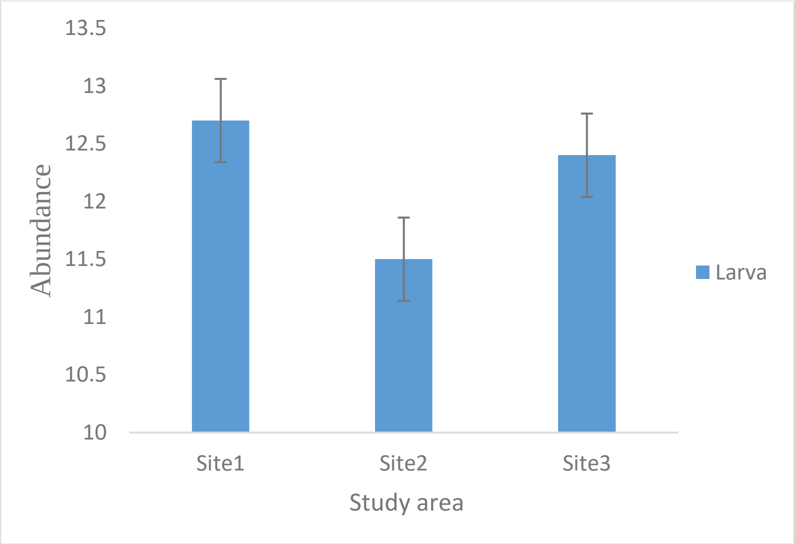

Figure 3. Abundance of larvae of *T. absoluta* (Mean and SD) in different study sites in Kathmandu Valley

4.2 Seasonal variation of *T. absoluta* larva in different sites of Kathmandu Valley

Tuta absoluta abundance varies seasonally. In each season, the mean abundance and standard deviation (SD) of *T. absoluta* are summarized in (Table 1). In site1, Tuta absoluta abundance fluctuates throughout the year, with the highest population levels observed during autumn (16.83±3.80), followed by summer (14.78 ± 3.79) and spring (10.83±2.27). Winter (7.17±2.27) shows the lowest abundance levels.

Table 2. Seasonal abundance of *T. absoluta* larva across the different sites in Kathmandu Valley

Season	Site1 (Dahachowk)		Site 2 (Gundu)		Site3 (Naikap)	
	Mean	SD	Mean	SD	Mean	SD
Spring	10.83	2.27	3.17	1.46	7.83	3.62
Summer	14.78	3.79	12.11	3.21	13.11	3.81
Autumn	16.83	3.80	21.00	2.58	17.83	7.60
Winter	7.17	2.67	9.33	4.42	6.00	1.29

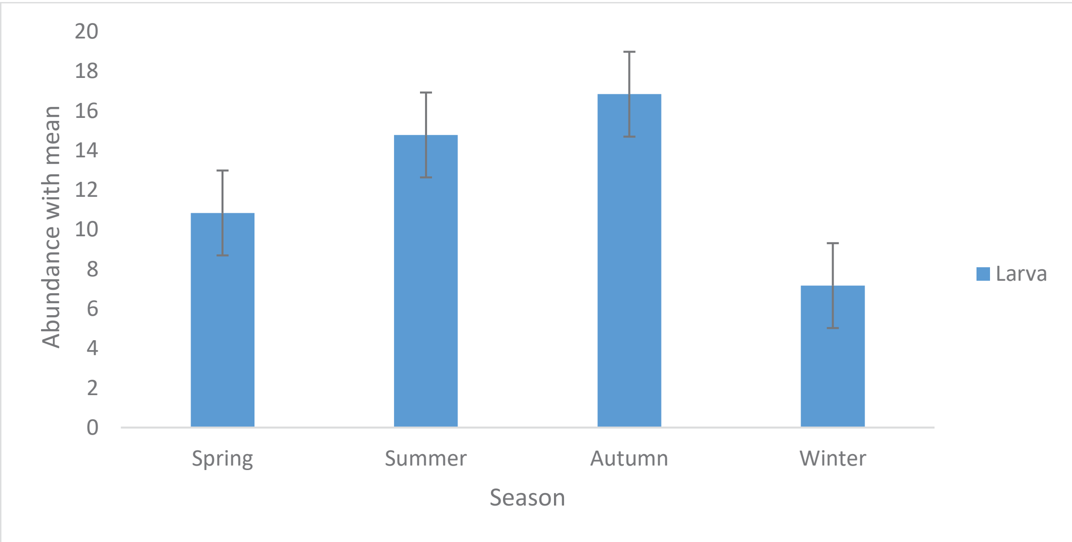

Figure 4. Seasonal abundance of T. absoluta at site 1, Dahachowk, Kathmandu Valley

At Site 2, *T. absoluta* abundance follows distinct seasonal patterns (Fig 2 and Table1). Autumn has the highest abundance, with an average of (21± 2.58) individuals. The second highest abundance is found during the summer, averaging (12.11 ±3.21). Over the winter, abundance declines to (9.33± 4.42). Finally, spring shows the lowest abundance, with an average of (3.17± 1.46).

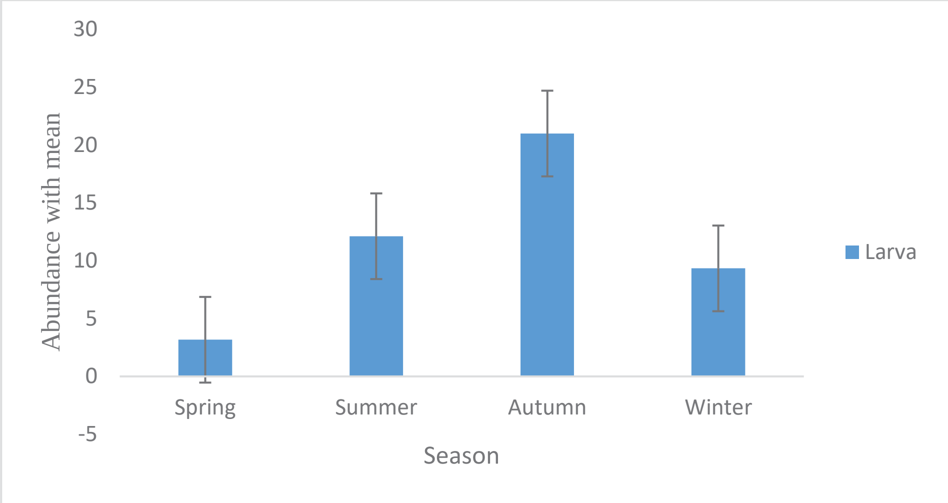

Figure 5. Seasonal abundance of *T. absoluta* at site 2, Gundu, in Kathmandu Valley

Tuta absoluta at Site 3 exhibits distinct seasonal patterns, with the highest abundance levels occurring during autumn, followed by summer, spring, and winter.

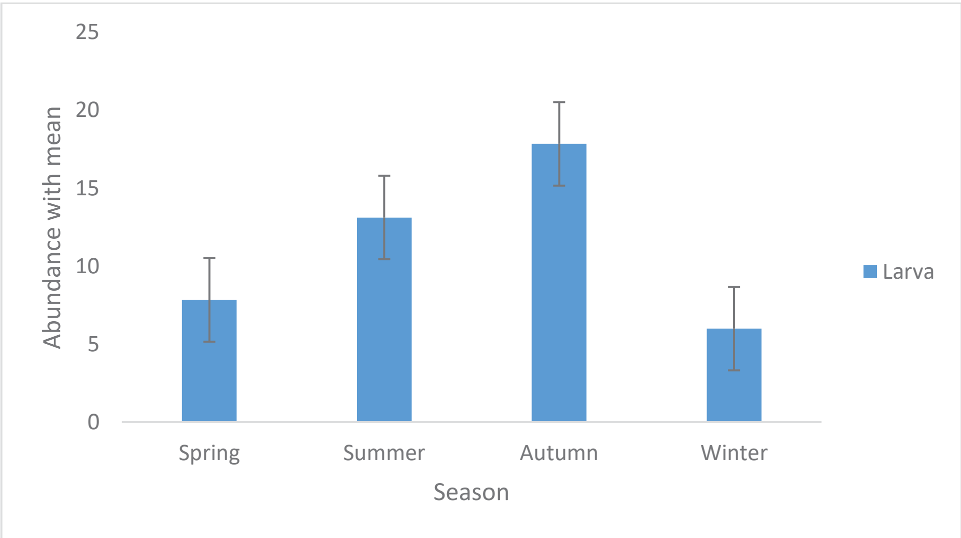

Figure 6. Seasonal abundance of *T. absoluta* at Site 3 Naikap Kathmandu Valley

4.3 Relation of *T. absoluta* larva with physical parameters

Generalized linear models (GLMs) showed there was no statistical association between *Tuta absoluta* abundance and temperature based on environmental parameter testing. On the other hand, humidity appears to be statistically significant with abundance at site 1. Similarly, at site 2, temperature and humidity have statistically significant effect on the dependent variable. Furthermore, in site 3, temperature and humidity both exhibit statistical significance with respect to the intercept and coefficients. It means that all three variables influence the dependent variable significantly.

Table 3. Generalized linear model (GLM) with Poisson distribution and log link function test showing the relations between *Tuta absoluta* abundance and environmental variable at different study site in Kathmandu Valley**.**

Dahachowk				
Variable	Estimate	Std. Error	Z- value	Pr(>|z|)
Intercept	2.082	0.377	5.529	0.000323***
Temperature	**-0.019**	0.010	-1.885	0.05948.
Humidity	0.019	0.007	2.825	0.00473**
Gundu				
Variable	Estimate	Std. Error	Z value	Pr(>|z|)
Intercept	3.625	0.489	7.419	0.000118***
Temperature	**-0.059**	0.016	-3.767	0.000165***
Humidity	0.012	0.006	1.855	0.0064***
Naikap				
Variable	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	3.159	0.514	6.151	0.000772***
Temperature	**-0.069**	0.017	-4.079	0.000453***
Humidity	0.024	0.007	3.301	0.000963***

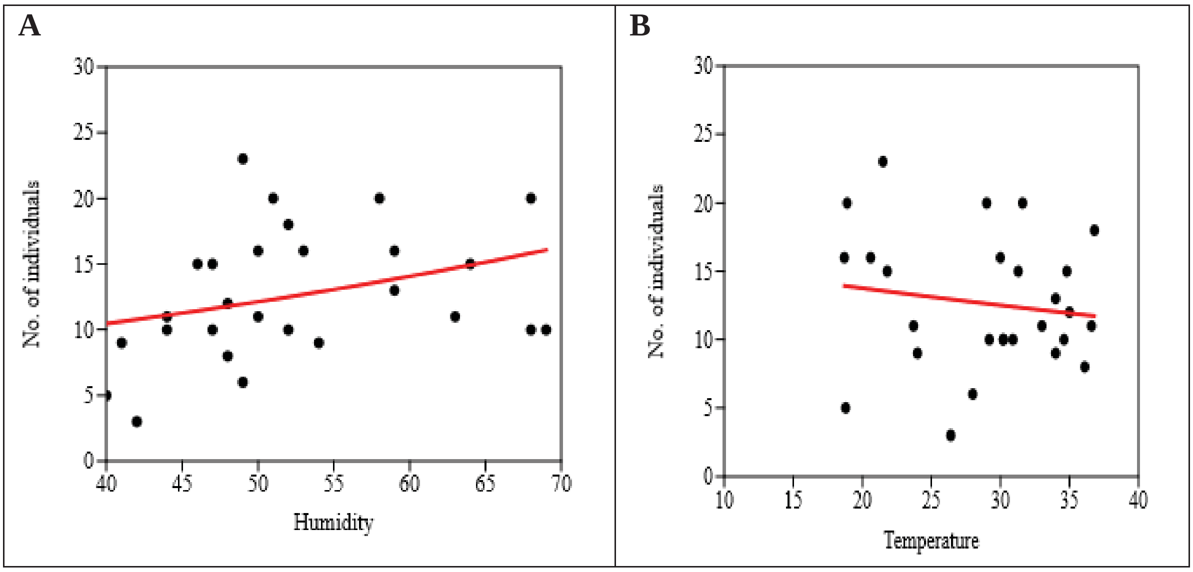

Figure 7. Relationship between A) No. of individuals and Humidity and B) No. of individuals and temperature in Dahachok kathmandu

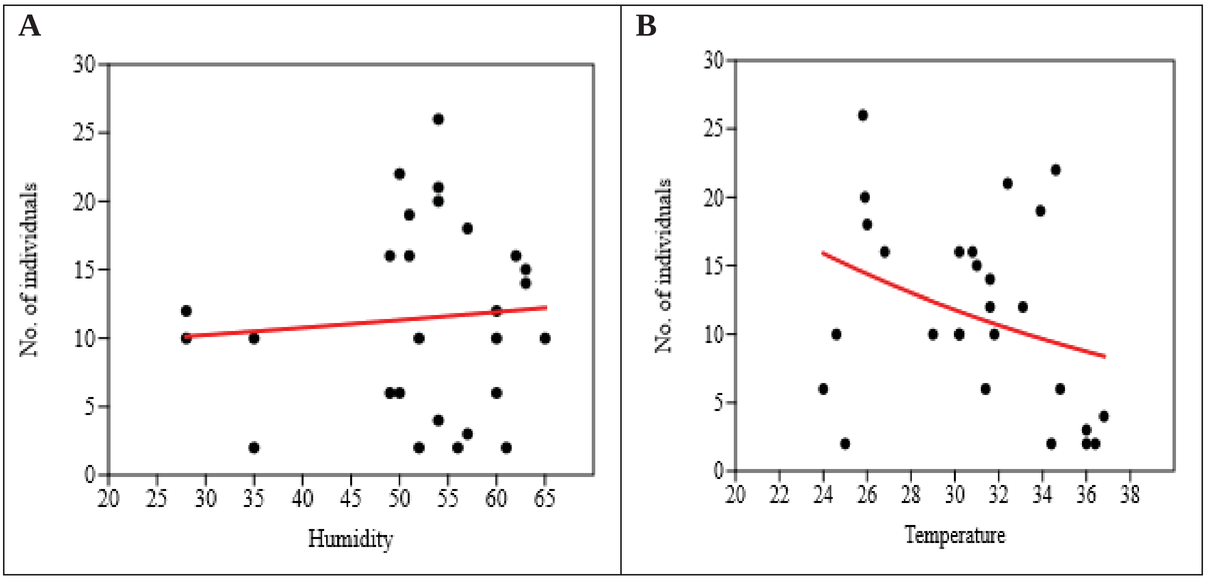

Figure 8. Relationship between A) No. of individuals and Humidity and B No. of individuals and temperature in Gundu Kathmandu

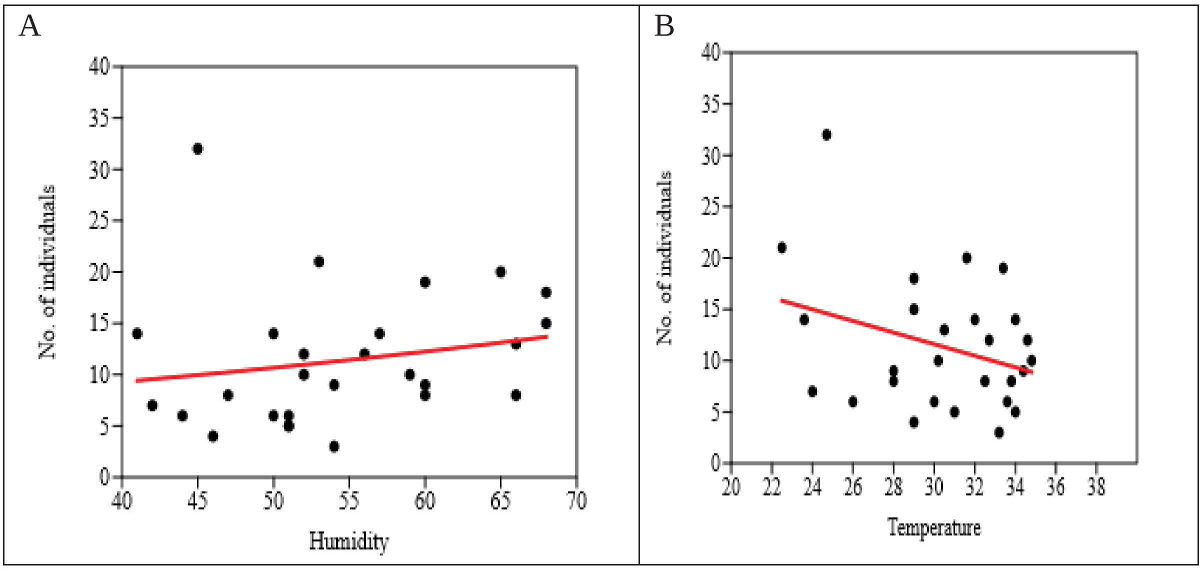

Figure 9. Relationship between A) No. of individuals and pH and B) No. of individuals and temperature in Naikap Kathmandu

5. DISCUSSION

5.1 Abundance of *Tuta absoluta* larva in different sites of Kathmandu Valley

The present study was conducted at three different sites in Kathmandu valley to study *T. absoluta* larvae distribution and abundance. Larval counts and means varied across the sites based on the data collected. The highest larval count in Site1, followed by site 2 and least in site3. In this study, only tomato plants were planted on site 1 i.e., intercropping techniques were not practiced resulting in higher abundance of larvae than other sites 2 and 3 where tomatoes were intercropped with coriander (*Coriandrum sativum L.*) and other vegetables. Similar results were revealed by (Joshi et al. 2018) stating that lesser population of *T. absoluta* larvae can be observed if tomatoes are interplanted with coriander and gallant soldier. Further supported by (Illakwahhi & Srivastava 2017) suggesting crop rotation with non-host plants such as crucifers should be emphasized and monocropping techniques should be avoided to minimize pest attack. At Naikap site, the soil is thoroughly treated with calcium oxide powder along with vermicompost and cattle manure and fields are left uncultivated for some time prior the tomato plantation along with complete removal of leftover residues from previous plantation comparatively showing lesser abundance of larva than other sites. Similar facts were stated by (Brevault et al.2014, Sylla et al. 2018, Adhikari et al. 2019) that the areas that strictly follows IPM strategies including physical, cultural control along with chemical methods showed lesser abundance of pest populations. Larval abundance variation might be due to resource availability, environmental conditions, natural enemies, and competition within or between species (Sylla et al. 2018). Population size and distribution are influenced by complex relationships between pest species and the surrounding ecosystem. The farmers at site 2 and 3 used chemical fertilizers like Abamectin benzoate, Dadaguard mixed with Azadiracthin oil to control T.absoluta larva whereas at site 1 more preference was given to Indoxcarb insecticides. Chemical insecticides like Spinosad, Abamectin benzoate, Triflumuron and Diafenthiuron are effective against *T.absoluta* (Joshi et al. 2018). Chemical fertilizers like Dadaguard along with Azadiracthin oil (neem oil) was found effective and showed a lower larval infestations of lepidopteran pest i.e., leaf eating caterpillar (Kattel et al. 2023). Chlorantraniliprole insecticides was found to be most effective in managing *T. absoluta* larva (Simkhada et al., 2018). There was a significant rise in egg and larvae abundance on tomato plants grown in intensive production mode, where pesticides are frequently used (Oliveira et al. 2023). Climate conditions play an important role in population dynamics of pest insects in several agroecosystems (Wallner 1987, Cocco et al. 2015, Bacci et al. 2019). Insects' physiological, behavioral, and morphological adaptations as well as population fluctuations have been strongly linked to host plant characteristics as well as climate factors (Singer & Parmesan 2010). During the course of plant development and growth, they undergo several changes in their nutritional and defensive traits, which affect their performance and population dynamics (Campos et al. 2006). In addition, the use of insecticides against this pest can also affect population dynamics by reducing pest populations or increasing them depending on resistance mechanisms (Guedes et al. 2019). The results suggest that *T. absoluta* infestations may vary based on population dynamics and ecological factors. In addition, the study examined the relationship between larval abundance and environmental variables, which may provide valuable insights into the factors affecting the pest's proliferation.

5.2 Seasonal abundance of *Tuta absoluta* larva in different sites of Kathmandu Valley

Tuta absoluta populations fluctuate at site 1, where abundance levels vary throughout the year. There are higher population levels in autumn, with a mean abundance of 16.83 ± 3.80, followed by summer (14.78 ± 3.79) and spring (10.83 ± 2.27). In contrast, winter is the season with the lowest abundance levels of *Tuta absoluta*, with a mean of 7.17 ± 2.27. In addition, the present study showed that *Tuta absoluta* populations fluctuate continuously throughout the year at Site 2. This is maximum abundance in autumn and minimum abundance in spring. Furthermore, *Tuta absoluta* abundance differs seasonality at site 3. A pest's abundance is greatest during autumn, followed by summer, spring, and winter. Tomato pest management strategies can be impacted significantly by seasonal variations in *Tuta absoluta* abundance. There seems to be a higher abundance of pests during autumn and summer, which could be due to more favorable conditions for development and reproduction (Sylla et al. 2018). In these periods, temperatures are typically higher and humidity is higher, which may contribute to the growth of pest populations (Tonnang et al. 2015). On the other hand, low abundance during winter may be expected due to a reduction in pest activity due to colder temperatures, which may disrupt the pest's life cycle (Biondi et al. 2018). There are also other factors that may relate to the observed seasonal patterns, such as the availability of host plants, natural enemies, and agricultural practices (Brévault et al. 2014, Sylla et al. 2018). For a more comprehensive understanding of *Tuta absoluta* populations in the studied locations, further investigation of these potential drivers of seasonal variation is warranted.

5.3 Relation of *T. absoluta* larva and physical parameters

The findings reveal that environmental factors have a significant impact on pest population dynamics. Temperature and humidity are both known as major factors affecting insect populations, but their effects vary based on location and context (Sylla et al. 2018). In this study, variations in climatic conditions and their interactions with pest populations were observed across the sites. The temperature showed negative effects on abundance in all three sites. The mean temperature are 28°C, 30.4°C and 30.9°C in site 1,2 and 3 respectively with higher abundance in site 1 and gradually decreasing in site 2 and 3. It is imperative that larvae be kept between 20°C and 30°C for optimum development as *T absoluta* reproduction occurs best between 15°C and 25°C (Martins et al. 2016). Optimum temperature for survival and development of immature stages ranges from 21-23°C (Mohammad et al.2022). The survival and development of entire immature stages is increased up to 25°C and start to decline as temperature increases (Dong et at.2019). Survival rates of *T. absoluta* population was obtained at temperature ranging from 23-27.5°C (Ozgokce et al.2016). Temperatures that are warmer promote the growth and reproduction of pests more rapidly (Krechemer & Foerster 2019). *Tuta absoluta's* life cycle can be accelerated by higher temperatures, and its population can grow more quickly thereby. Temperatures that are too high or too low can also affect pest survival(Krechemer & Foerster 2019). In order to implement appropriate control measures for *Tuta absoluta*, it is necessary to monitor fluctuations in temperature (Martins et al. 2016).

The humidity showed positive effects on larvae population in all the three sites. *Tuta absoluta's* reproductive capacity and survival are affected by humidity levels (Guimapi et al. 2016) . The presence of high humidity can create favorable conditions for pest development and increase the number of pests (Saad et al. 2020). *Tuta absoluta* eggs, larvae, and pupae prefer moist environments to survive (Guimapi et al. 2020). Furthermore, humidity influences pest dispersal and the ability of pests to locate suitable host plants (Tadele & Emana 2017). It is necessary to monitor and manage humidity levels in tomato growing areas in order to control the infestation of *Tuta absoluta*. A number of factors facilitate the spread of the pest, including high temperatures, high relative humidity, and a high tomato production rate (Guimapi et al. 2016). In order to gain a comprehensive understanding of pest dynamics, multiple environmental factors should be considered rather than focusing on just one.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In the Kathmandu Valley, *T. absoluta*, an invasive pest that affects tomatoes, shows varying abundance levels based on site and season. Among the three sites, site 1 had the highest abundance, followed by site 2 and site 3, with autumn showing the highest infestation levels and winter the lowest. Environmental factors such as temperature and humidity were also investigated in relation to *T. absoluta* abundance. Site 1 showed a significant effect of humidity on pest abundance, whereas site 2 showed a significant impact of temperature on pest abundance. The populations of *T. absoluta* were statistically affected by both temperature and humidity at Site 3. In this study, the distribution and abundance of *T. absoluta* in the region are explored in detail. This supports the need for tailored integrated pest management practices in different sites based on their specific environmental conditions. In order to sustain tomato production and minimize the impact of this invasive pest on the Kathmandu Valley agricultural community, these insights are critical.

6.2 Recommendations

- Insecticides resistance to *Tuta* larva should be studied as it plays a vital role in abundance of tomato leaf miner population.
- Effects of other environmental variables such as soil status, rainfall, host plant availability should be thoroughly analyzed.

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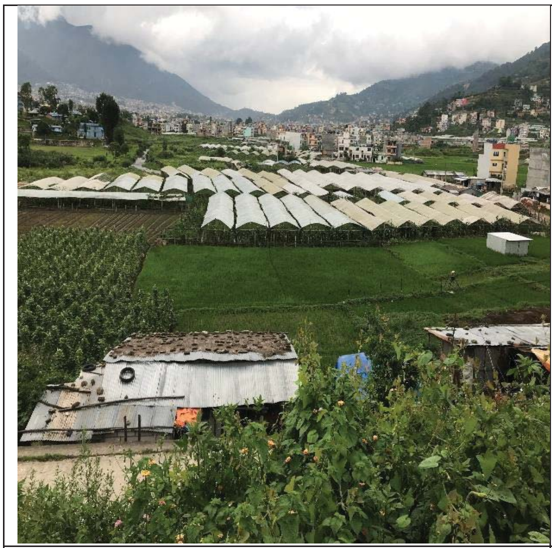
PHOTO PLATES



Dahachowk site

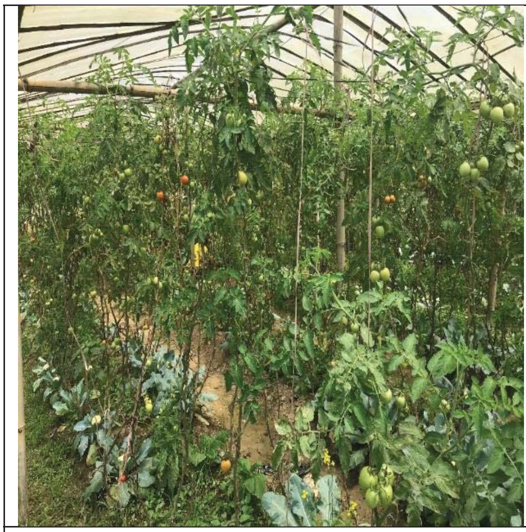
Naikap site

Larvae with infested leaf

Brushing off larvae from leaf

Tuta larvae

Collecting samples



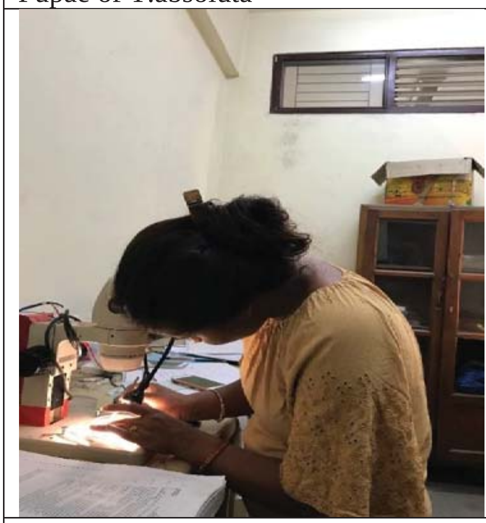
Sampling sites

Sampling sites

Pheromone trap for Tuta absoluta

Larvae infesting apical part

Adult mating of Tuta absoluta

	
Eggs of T.absoluta	Larva of T.absoluta
	
Pupae of T.absoluta	Adult of T.absoluta
	
Lab work	Hygrometer showing temperature and humidity data

	
Sampling vials	Sampling vials