STUDY ON CITRUS TRISTEZA VIRUS (CTV) VECTORS IN A MANDARIN FARM OF KAVREPALANCHOWK DISTRICT, NEPAL

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A thesis submitted in partial fulfillment 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
Kirtipur, Kathmandu
Nepal
November, 2017

DECLARATION

I hereby declare that the work presented in this thesis entitled "**Study on Citrus Tristeza Virus (CTV) Vectors in a Mandarin Farm of Kavrepalanhowk District, 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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RECOMMENDATION

This is to recommend that the thesis entitled "**Study on Citrus Tristeza Virus (CTV) Vectors in a Mandarin Farm of Kavrepalanhowk District, Nepal"** has been carried out By Mr. **Pramod Acharya** for the partial fulfillment of Master's Degree of Science in Zoology with special paper **Entomology** This is his original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

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जि.प्र.का. काठमाडौं द.नं.:४९८/०४७/०४८
स.क.प. काठमाडौं द.नं.: ३८८/०४८
पान नं.: ३००९७९५५४
ठेगाना: अमरावती मार्ग, कोटेश्वर
पो. बक्स नं.: ८११५, काठमाडौं, नेपाल
फोन नं.: ९७७-१-५१४७१६६
फ्याक्स नं.: ९७७-१-५१४७०९०

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This thesis work submitted by Pramod Acharya entitled "**Study on Citrus Tristeza Virus (CTV) Vectors in a Mandarin Farm of Kavrepalanchowk District, Nepal**" has been accepted as a partial fulfilment for the requirements of Master's Degree of Science in Zoology with special paper Entomology.

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ACKNOWLEDGEMENTS

I would like to express my heartful gratitude to my supervisor Dr. Dayaram Bhusal, Associate professor, Central Department of Zoology, T.U. Kirtipur for his supervision, guidance and invaluable suggestion throughout my study. I am thankful to my Co-supervisor Dr. Chiranjivi Regmi, Vice - Chairperson, ECARDS-Nepal for his guidance and suggestion throughout my study. I am thankful to University Grants Commission (UGC) for providing Master's thesis support grants. I am thankful to our honorable Head of Department Prof. Dr. Ranjana Gupta, Central Department of Zoology, T.U. Kirtipur for providing me such an opportunity to carry out this dissertation work. I am indebted to my friends: Rashmi Paudel, Prashant Neupane, Sandesh Gurung, Pujan Adhikari and Praabin Baral for their constant help during study period. Heartly thanks to my family without which this work is impossible to complete. I also acknowledge to all the teachers, friends and staffs of Central Department of Zoology for their continuous aspiration and motivation.

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LISTS OF ABBREVIATIONS

CTV	:	Citrus Tristeza Virus
GLM	:	Generalized Linear Model
PCA	:	Principal Component Analysis
DA	:	Discriminant Analysis
HH	:	Higher Humidity
MH	:	Moderate Humidity
LH	:	Lower Humidity
HT	:	Higher Temperature
MT	:	Moderate Temperature
LT	:	Lower Temperature
S	:	South
N	:	North
W	:	West

ABSTRACT

Globally the pathogen of tristeza disease is transmitted by different species of aphids. Among them *T. citricida* and *T. aurantii* are two potential CTV vectors in Nepal. The present study was conducted to study the CTV vectors in mandarin farm of Kavrepalanchowk district, Nepal. The study was carried from August 2016 to April 2017. Data collection was carried out using yellow trap method. The local temperature and humidity of the farm was recorded with the help of alcohol thermometer and hygrometer respectively. *Toxoptera citricida* and *Toxoptera aurantii* were found potential aphid vectors for virus transmission. Among identified species, *T. citricida* abundance was higher (90%) than *T. aurantii* (10%). In the present investigation, abundance of aphids was found to be affected by local temperature, humidity, aspects and seasons. The abundance of aphid species was correlated with temperature ($p<0.05$) similarly negative relation was obtained between humidity ($P<0.05$) and abundance of CTV vectors. Minimum abundance of aphids were recorded during winter season ($P<0.05$). These results highlight effects of bioclimatic parameters on distribution and diversity of CTV vectors in Citrus farm of Kavrepalanchok district, Nepal.

1 INTRODUCTION

1.1 Background

Nepal is an agricultural country having 66 percent people directly engaged in farming. This sector is contributing nearby 33.6% to the GDP (MOAC, 2010). Moreover, citrus contributes 14% to the total agricultural gross domestic product (NARC, 2000). The term citrus fruit covers a large range of fruits of Rutaceae family including mandarin (*C. reticulata*). Citrus fruits are cultivated all over the world in tropical and subtropical region where there are suitable soil and climatic condition (Saunt, 1990). In Nepal, the climatic condition of mid-hill regions having altitude range of 800 m to 2100 m from east to west of the country are considered favorable for all types of citrus fruit cultivation (Lama, 1988). The three most important species on citriculture in Nepal are mandarin (*C. reticulata*), sweet orange (*C. sinensis*) and acid lime (*C. aurantifolia*). Among them mandarin takes 1st rank, sweet orange 2nd rank and acid lime 3rd rank in term of area coverage and production (Regmi, 1982). The history of citrus fruit cultivation in Nepal is not well documented but the description of fruits in old scriptures about their importance in religious ceremonies and medicinal values indicates that citrus farming must have been traditional practices since ancient period. However commercial cultivation of citrus in Nepal started only after 1970 (NCRP, 2010). Mandarin is one of the most important commercialized and nutritional fruit. The total production of citrus in Nepal is around 88699 tons with an estimated productivity of 10 mt/ha. Mandarin is priority cash generating commodity of mid hill farmers and is estimated to cover about 60% area of total citrus growing area of Nepal (NARC, 2000). At present major mandarin producing districts of Nepal are Illam, Panchthar, Terathum, Dhankuta, Bhojpur, Sindhuli, Ramechap, Kavrepalanchok, Dhanding, Gorkha, Lamjung, Tanahun, Kaski, Shayanja, Gulmi, Arghakhanchi, Dailakh, Dadeldhura, Baitadi and Darchula (Shah, 1971).

Since last ten years area and production of mandarin fruits has increased by more than 2 folds whereas increase in productivity is very slow. The productivity of mandarin fruits in Nepal is very low (10.8 mt/ha) as compared to 20-50 mt/ha in most mandarin growing countries of the world (NCRP, 2009). Thus the increase in total production of mandarin in Nepal is primarily attributed to the increase in area under cultivation. So there is enormous scope of increasing productivity of mandarin fruits crops in Nepal, which can be achieved by utilizing better varieties along with improved orchard management practices (NCRP, 2010). Mandarin trees can be cultivated using varieties of ways, such as seed budding and grafting (Knoor and Shah, 1971). However, these great trees are very susceptible to many diseases and pests. More than 30 virus diseases of citrus have been reported in the world (Yokomi and Damsteegt, 1991). Diseases like tristeza, citrus greening disease, citrus canker and citrus variegated chorosis are specifically bad (Rana and Sharma, 1965). The tristeza in particular is one of the most devastating diseases causing extensive damages to citrus trees.

Tristeza is a serious disease of mandarin caused by citrus tristeza virus (CTV). Natural spread of CTV is primarily through propagation of infected bud wood and by aphid vectors. CTV is not seed-borne (Wallece, 1978). CTV is a member of the closterovirus group and is considered as one of the most economically important pathogen virus of citrus (Bar joseph *et al*., 1992). CTV causes tree decline grafted on orange and its species. It causes multiple disease syndromes in citrus trees. The pathogen of citrus tristeza virus (CTV) is transmitted by different species of aphids, including *Toxoptera citricida*, *T. aurentii*, *A. gossypii* and *A. spiraecola* (Yokomi and Damsteegt, 1991). Among them *T.citricida* is an efficient virus vector (Bove, 1977). *T. citricida* is thought to have originated in south east Asia (Kirkaldy , 1907 and Rocha-Pena *et al*.,1995) and is common throughout Asia, including China , Cyprus , India , Nepal, Taiwan , Vietnam, etc. The disease in lime (*C. aurentifolia*) vectored by aphids, *T. citricida* and *T. aurantii* were reported for the first time in Nepal from Pokhara (Knoor and Shah, 1971) ,which has extended throughout Nepal from east to west (Tomiyasu and Verma 1999).

CTV is semi-persistently transmitted by several aphid species (Bar-Joseph and Lee, 1989). Aphid vectors acquire virus from an infected tree with feeding times ranging from 5 min to hours. The transmission efficiency of the vector increases as feeding times are increased up to 24 hours. Aphids remain viruliferous for 24-48 hours after feeding on infected plants. Many aphid species that feed on an infected citrus tree can acquire CTV, as detected by ELISA (Cambra *et al*., 1981), but only a few species can transmit it to new plants. *T. citricida*, the most efficient vector, exists often most abundantly on citrus trees (Yokomi *et al*., 1994). There are different strains of CTV in different countries but in Nepal two types of CTV strains, stem pitting and vein clearing CTV strains are reported from Pokhara. Most strains of CTV, including severe stem pitting strains, are effectively vectored by *T. citricida* (Regmi and Lama, 1992). *T. aurantii* can transmit some CTV strains less efficiently than *T. citricida* (Hermoso de Mendoza *et al*., 1984). *A. gossypii* can transmit some strains efficiently and is the most important CTV vector in regions where *T. citricida* is not present (Hermoso de Mendoza *et al*., 1984; Roistacher *et al*., 1991; Yokomi *et al*., 1994 and Ballester-Olmos *et al*., 1993).

Excellent quantities, highly commercial, cultural and ecological values of the mandarin cultivation have been rapidly expanded. Large numbers of mandarin trees are observed CTV affected at Pokhara, Gorkha and Lamjung (1000m) were affected by CTV (Tomiysu and Verma, 1999). TPresent Scenario suggests that, citrus trees at farm of Kavrepalanchok may also be attack of CTV in the long run.

1.2 Objectives

1.2.1 General objective

To explore the CTV vectors in mandarin farm Kavrepalanchok district, Nepal.

1.2.2 Specific Objectives

- To identify CTV vectors in selected mandarin farm.
- To find out the seasonal abundance of CTV vectors in study sites
- To compare the effect of physical parameters (temperature, humidity and aspects) on CTV vectors
- To study the monthly variations of CTV symptoms.

1.3 Significance of study

Selected farm is one of the most important citrus growing area of Kavrepalanchok district. It lies in mid hill agro ecological zone of Nepal, where no research has been carried out on the citrus tristeza virus and its vectors. Recently huge mandarin production is impaired due to the spreading CTV in mid hill agro ecological zone located districts of Nepal such as Lamjung, Dhankuta, Kavrepalanchok, Gorkha, Kaski, and Sindhuli. The actual reason of outbreak of this disease in these areas is still unclear. Therefore, exploration on CTV vectors and their population structures are urgent to be studied in Nepal. Thus study was carried out to find the population of aphids vector of CTV and its relation with season, prevailing temperature, humidity and morphological characteristics of mandarin plants. Theanticipated results of this study hopefully will provide minimum baseline to monitor the CTV and its vector in the citrus orchards.

2 LITERATURE REVIEW

Like many other crops, citrus has many pest and disease problems, among them the important one is Aphid pest and typically vector of citrus tristiza virus (CTV) namely *T. citricida* and *T. aurantii* are serious ones in the citrus orchrds in the world including Nepal.

Regmi *et al.* (2000) made field survey on lime trees of different farms of Nepal and found infected with CTV. Chatterjee *et al.* (2000) carried out multiple correlation studies to access the influence of important weather factors on the population fluctuation of insect pest of mandarin orange (*C. reticulata*) at Pedong, West Bengal during 1966-1997. Garcia *et al.* (2000) sampled 15 trees per orchard and infested shoots were collected from three strata of the tree height. Aphid species found were *A. gossipyii, A. spiraecola, A. fabae* and *T. aurantii* in a contagious distribution, Medium stratum was most preferred. Michaud and Belliure (2000) conducted the field study in which the colonies of *T. citricida* were initiated with different number of foundress on ungrafted citrus tree. It was found that the growth rate, longevity of foundress and size of *T. citricida* colonies were all positively correlated with numbers of foundress. Colony initiated by many foundress produced alatae earlier than those initiated by more foundress and the longevity of foundress was affected by their number but foundress fecundity and nymphal survival decreased as foundress number increased. Tang *et al.* (1999) compared the development, reproduction and population growth parameter of the brown citrus aphid, *T. citricida*, and were evaluated at 25^0C on 5 citrus related host plants. Further *T. citricida* had longer nymphal development times and adult reproductive period at 30^0C compared with at 25^0C and 30^0C. Tsai and Wang (1999) also studied the life table of brown citrus aphid at different temperatures. It was observed that the optimal range of temperatures for *T. citricida* population growth was 20^0C- 30^0C. Agarwala and Bhattacharya (1995) carried out the seasonal performance in the colonization of its hosts. Individual aphids of these species suffered greater mortality during development and then intrinsic rate of increase slowed down. The origin of *T. citricida* is thought to be in south East Asia (Kirkaldy, 1907; Rocha-Pena *et al.,* 1995). It has been reported in the Pacific Region including Nepal, China, Taiwan, India, Japan, Thailand, Malaysia, Sri Lanka, etc. Kuzumi and Kuhara (1992) recorded the ability of *T. citricida* to transmit the tristeza in Spain. Rostacher *et al.* (1991) recorded abundance of CTV and its prime vector in different countries like China, India, Thailand, Malaysia, Philippines, Hawaii, South Africa and Australia mainly because of transportation of citrus materials from American countries. Lastra *et al.* (1991) recorded CTV in Costarica but did not absence *T. citricidus* in the field tree, whereas *T. aurintii* was found in abundance. Rostacher (1989) recorded the number of pterygote aphids directly related to the CTV infection in field trees. Bhumannavar *et al.* (1989) carried out the study on relative susceptibility of 78 varities of hybrid citrus to the incidence of black citrus aphid, under tropical humid condition in South India. It was observed that none of the varieties was completely free form aphid incidence. Regmi (1982) made field survey on lime trees of horticulture research Station at Pokhara and Sanothimi (Bhaktapur) and found infected with CTV. Roistacher *et al.* (1980) and Roistacher (1981) reported that *T. citricida* was highly efficient vector of tristeza in Riverside California in control condition. (Roistacher, 1981) found that 46 field isolates of CTV and CTV-ST were transmitted at 100 per cent efficiency by *T. citricidus* from sweet orange acquisition host plants Mexican lime indicator aphids.

Bar- Jopesh *et al.* (1977) recorded that transmission of CTV by aphid vector depends upon the readiness of the aphid population to colonize in the host citrus plants. Pujal *et al.* (1972) found that there was the high positive correlation between trap plant colonization of *T. citricidus* and tristeza infection rate. Greening and tristeza are most important diseases responsible for citrus decline in Nepal since 1964 (Knoor and Shah, 1971). Catling (1970) reported the presence of greening vector insect *Diaphorina citri* in Pokhara, Nepal. Some on citrus greening disease (Regmi, 1982) and natural spread of citrus tristeza virus in Nepal (Regmi and Lama,1982) have appeared. Knorr (1970), Schwarz (1965), and Shah (1971) supposed naturally spread CTV is to be carried out by the aphid vectors. *T. citricidus* and *T. aurintii* probably account for the spread of CTV disease in the field. Simanton and Knoor (1969) analyzed the monthly records of aphid population in 130 citrus groves in Florida. *A. spiraecola*, *A. gossypii* and *T. aurantii* were the only aphid species attacking citrus trees. All three species were known to transmit virus. Sharma (1968) reported the presence of *Toxoptera citricidus* and *T. aurintii* in Nepal. The natural movements of aphids from one tree to immediate adjacent trees or at longer distance will depend on additional factor such as attractiveness of a given citrus cultivar for aphid alightment (Nature *et al*., 1968) Rana and Sharma (1965) reported the national pest status of 14 types of aphidsin crops for the first time.

Capoor and Rao (1967) also identified *T. aurantii* as a virus transmitter. They recorded three distinct strains viz. severe strain, mid strain and seeding yellow strain of the CTV with their characteristics behavior in Indian mandarin and kagati lime. Capoor (1963) recorded that tristeza was neither transmitted by simply the mechanical sap inoculation through seed nor through the grafting tools. Capoor (1961) recorded distinctive chronic spots and dashes along to the lateral veins within 40 days after virus inoculation due to of the virus. So, the stem pitting symptom was noticed in the citrus plants. Knoor *et al.* (1960) recorded over thirty millions of mandarin trees to have been destroyed in Florida by tristeza and its prime *T. citricida*. Vasundara *et al.* (1960) recorded the establishment of *T. citricida* aphid as vector of tristeza virus. Schwarz (1965) recorded same disease from Africa, and Dickson (1953) recorded from California in the citrus plants. Dickson and Flock (1999) conformed presence of tristeza virus in Maharastra state (Nagpal) India. Graphids *et al.* (1959) found vector transmission of tristeza might occur at any time of the year, with the exception of period of hot and dry weather during summer. Schneider (1959) recorded reduced sieve tubes and phloem vessels due to tristeza infection. Experimental evidence on tristeza was first obtained during (Vasudeva and capoor, 1958). Mendit *et al.* (1954) recorded dead sour orange due to CTV in Venezuela that proceeded rapidly in five million plants in 1987. Costa *et al.* (1968) recorded transmission rate of *T. citricida* was more than 50 percent in California. Costa *et al.* (1949) showed transmission rate of 50-70 percent from sweet orange from mandarin Brazil. They found lemon as poor host of CTV. There was no report of transmission of CTV from lemon to lemon. Acromonte (1940) reported that colony of the CTV vector increase with increasing of temperature of the environment.

3 MATERIALS AND METHODS

3.1 The Study Area (Fig. 1)

Kavrepalanchowk is one of 75 districts of Nepal, near to the Kathmandu valley. It is bordered to the east by Ramechhap and Dolakha, west by the Kathmandu valley, north by Sindhupalchok and south by Sindhuli and Makawanpur. It lies on the geographical coordinates of 27°64' 54'' N latitude and 85°24' 44'' E longitude. The temperature usually ranges between 8^0C to 35^0C with an average annual rainfall of 2400 mm. There is exclusively agricultural diversity in Kavrepalanchok district due to the prevalence of a wide range of climatic and topographical variations. Citrus is the most important fruit crop of Kavrepalanchok that can bring economic change in the locality. Because of appropriate geography and climate, citrus is widely grown throughout this district.

Figure 1. Map of Study Area

3.2 Materials

i. Yellow Trap (380 cm^2)
ii. Camel hair brush (10A)
iii. Vials (5 ml)
iv. Microscope
v. 10A brush
vi. 10X hand lens
vii. Plastic rope
viii. Alcohol Thermometer
ix. Hygrometer
x. 70% Alcohol
xi. DPX
xii. Slides
xiii. Coverslips

3.3 Field design

Selected field, a Citrus farm of Kavrepalanchowk was categorized into three plots with respect to north, south and west aspects though bioclimatic parameters may differ with these aspects. Three mandarin trees were randomly selected from each plot to place yellow trap for the collection of CTV vectors.

3.4 Methods

3.4.1 Sampling methods

3.4.1.1 Yellow trap method

In this method the yellow trapping bowl, $2/3^{rd}$ filled with clean water was placed randomly in selected tree (Sharma, 1968). Attracted aphids launched on the water surface and were trapped. Trapped aphids were collected by using dry soft camel hair (10A) brush. Collected aphids in every 9 plants were separately preserved in separate vials containing 70 per cent alcohol. Collection period was nine months (August, 2016 – April, 2017).

3.4.1.2 Collection of bioclimatic parameters

For the collection of bioclimatic parameters (temperature and relative humidity) alcohol thermometer and hygrometer were placed along with the yellow trap. Collected data were clustered for further analysis. In our design temperature was classified as higher temperature (HT) ($\geq 25^0$C), moderate temperature (MT) (21^0C-24^0C) and lower temperature (LT) ($\leq 20^0$). Similarly, Relative humidity was also classified as higher humidity (HH) (≥60%), moderate humidity (MH) (50% -59%) and lower humidity (LH) (≤49%). The collected data of temperature and relative humidity were converted to nearest whole number.

3.4.2 Laboratory process

Collected aphids in every 9 plants were separately preserved in separate vials and taken in the lab of Central Department of Zoology and prepare their permanent slides. Aphids were identified by using a key (Roistacher, 1991).

3.4.3 Identification

Microphotography of all the taxonomic important organs of CTV vectors was done in the microphotography cell of Central Department of Zoology. The collected aphids were identified with taxonomic keys (Roistacher, 1991).

3.4.4 Taxonomic identification Key (Figs. 2,3 and 4)

Taxonomic Identification keys (Roistacher, 1991)

1. For both alate and apterygote aphids *T. citricida* approximately 10% larger and anal segment possesses large number of hair like filamentous structure than that of *T. aurantii* (Fig. 4).
2. On the alate forms only the third antennal segments for *T. citricida* are distinctly and entirely black whereas the same segments of *T. aurantii* are transparent or light colored (Fig. 3).
3. The pterostigma sector of the fore wings of *T. citricida* is light transparent brown compared to the distinctly dark to black colour in for *T. aurantii* (Fig. 2).
4. Median vein is always once branched in *T. aurantii* but is branched twice in *T. citricida* (Fig 2).

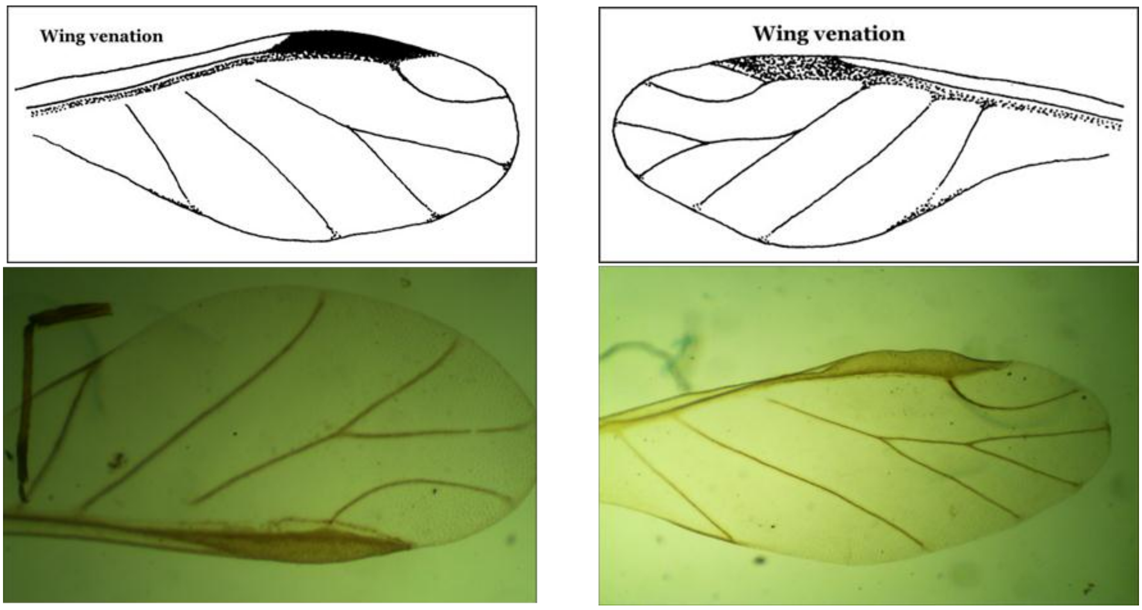

Fig. 2 Wing venation of *T. aurantii* (left) and *T. citricida* (Right)

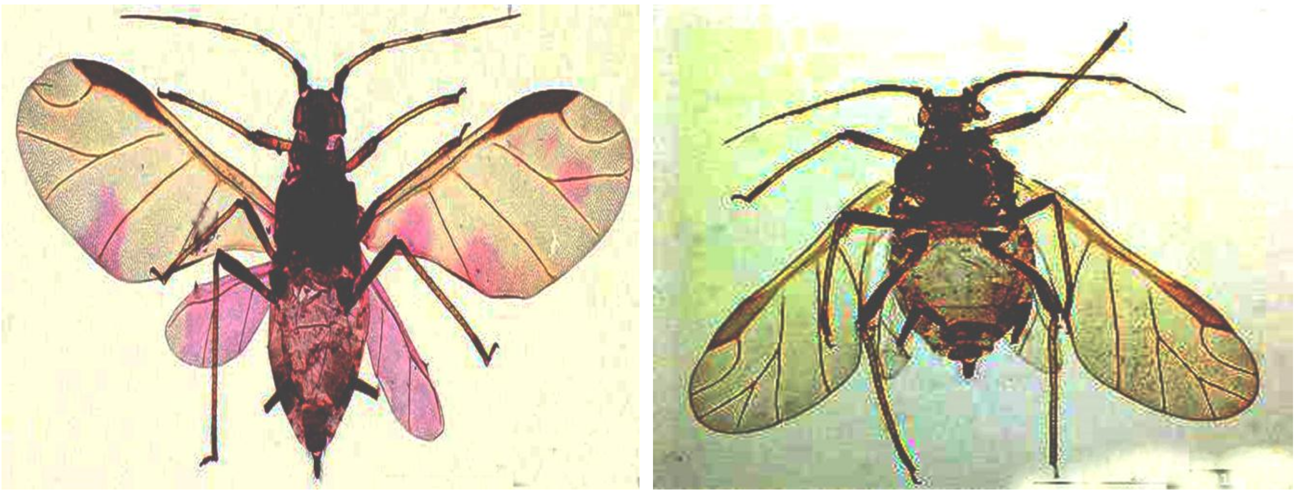

Fig. 3: ***T. aurantii*** **(left) and** ***T. citricida*** **(Right)**

Fig. 4 Cauda of ***T. aurantii*** **(left) and** ***T .citricida*** **(Right)**

3.5 Identification of citrus tristeza virus (CTV) symptoms

Citrus trees were carefully inspected for the visual symptoms of CTV such as stem pitting and vein clearing and data were recorded (Regmi and Lama, 1992).

3.6 Data analysis

The data on CTV vectors obtained from this study was analyzed with physical parameters such as temperature, humidity and seasons. The significant difference was tested by applying Generalized Linear Model with these parameters. Other descriptive analysis was carried out to show species composition and month-wise CTV symptoms with the help of Pie chat and Bar diagram respectively. Statistical analysis was performed by using “R” version 3.4.2 software packages.

4 RESULTS

4.1 Species Composition of CTV vectors

Altogether 2676 aphid specimens were collected form 9 sample trees of citrus farm Kavrepalanchowk. Highest numbers of aphid specimens were recorded in the month of August and least numbers of aphids were found in the month of January. Out of 2676 aphid specimen collected, 2439 (91.14%) comprise of *T. citricida* and rest 237(8.86%) of *T. aurantii*. (Fig 5 and Table 1).

Table 1 Abundance of aphid vectors (*T. citricida* and *T aurantii*) found in Kavrepalanchowk Citrus Farm

Months	***T. aurantii***	***T. citricida***	**Total aphid specimens**
August	36	367	**403**
September	32	334	366
October	35	342	377
November	35	389	424
December	23	264	287
January	20	180	200
February	22	232	254
March	34	331	365
Total	237	2439	2676

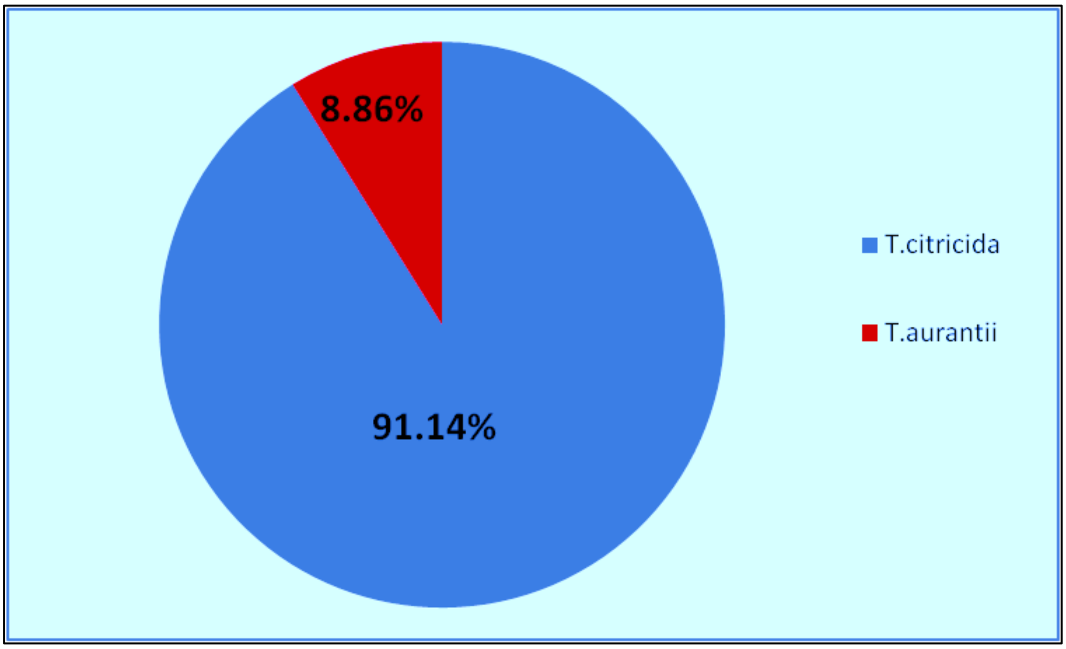

Fig. 5 Composition of Aphid Species

4.2 Effects of physical parameters on total abundance of aphids

Physical parameters are key factors to affect the distribution and diversity of many species including insects. Aphid's abundance is also significantly affected by various physical parameters. In this study temperature and humidity seemed major climatic factors to affect the diversity and abundance of aphids. The result of this study showed temperature has significant impact on abundance of aphid population. Number of aphids increased when temperature was more than 25^0 C ($P<0.05$) and decreased at lower temperature ($P<0.05$) (Fig 6 a). Like temperature humidity also showed significant effect on total number of aphids. This study showed aphid populations were affected by lower humidity. Populations were decreased in high humid air whereas less humid air was most favorable for citrus aphids (Fig 6 b).

Insect's diversity and abundance were greatly varied with seasons. During this study abundance of aphid population was significantly affected with winter season ($P<0.05$). In citrus farm of Kavrepalanchowk, population was found minimum in winter season but in spring and summer season maximum aphids were trapped and high population was noted on these seasons (Fig 6 c).

Table 2 Result obtained from Generalized Linear Model (GLM) of total abundance of aphids with variables (temperature and relative humidity, seasons) (GLM formula =Total abundance ~ relative humidity + seasons+ temperature, family = poisson (log)

Variables	**Estimate**	**Std. Error**	**Z-test**	**P-Value**
(Intercept)	2.67021	0.053553	49.861	**0.001**
Temperature(LT)	-0.43631	0.088028	-4.956	**0.003**
Temperature(MT)	-0.2278	0.048477	-4.699	**0.006**
Humidity (LH)	0.161343	0.089946	1.794	0.072
Humidity (MH)	0.029345	0.057939	0.506	0.615
Season (SP)	0.029432	0.064604	0.456	0.687
Season (SU)	0.044045	0.061671	0.714	0.451
Season (WI)	-0.13177	0.05975	-2.205	**0.027**

(Significance <0.05)

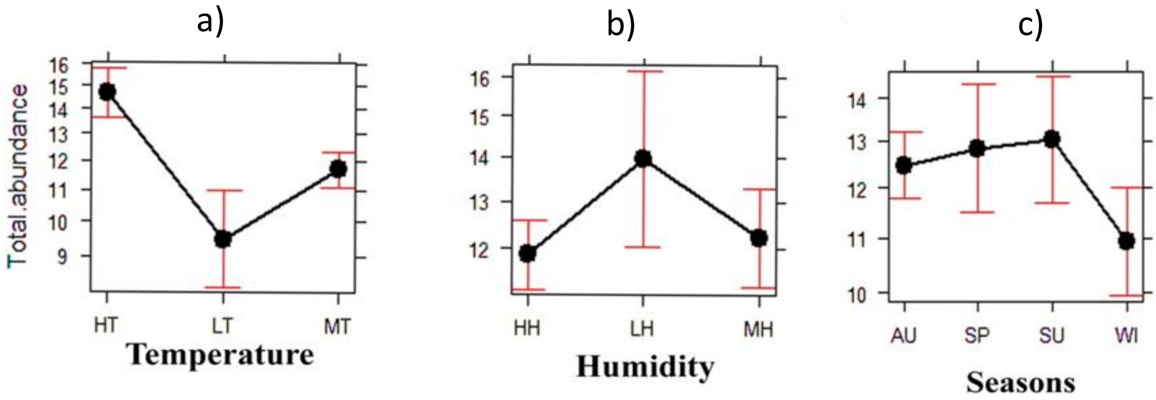

Fig 6 : Effect of different variables on total abundance of aphids. a) effect of temperature on total abundance of aphids b) effect of humidity on total abundance of aphids c) effect of seasons on total abundance of aphids.

4.3 Effects of physical parameters on *Toxoptera citricida*

The abundance of *T. citricida*, major vector for CTV transmission was affected by various physical parameters. Population of *T. citricida* changed with the changes in temperature, humidity and seasons. The abundance of *T. citricida* was found significantly affected by lower and moderate temperature ($p<0.05$) (Table 3). In the lower temperature the population of *T. citricida* was found minimum and population was increased as temperature increased and maximum population was encountered in higher temperature (Fig 7 c). Population abundance of *T. citricida* was also found affected by humidity. In this study population abundance of *T. citricida* was found high in low humid air and low in high humid air (Fig 7 a). Generalized linear model showed *T. citricida* was extensively significant with winter season ($p<0.05$) (Table 3). Winter season was found to be adverse for the *T. citricida* and minimum population abundance was recorded but in other seasons population of *T. citricida* was almost equal (Fig-7 b).

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Table 3 Result obtained from GLM of *T. citricida* with variables (temperature, relative humidity and seasons) (GLM formula = *T. citricida* ~ relative humidity + seasons + temperature, family = poisson (log)

Variables	**Estimate**	**Std. Error**	**Z-test**	**P- value**
(Intercept)	2.561074	0.05637	45.433	**0.002**
Humidity (LH)	0.114267	0.094767	1.206	0.227
Humidity (MH)	0.00707	0.060992	0.116	0.907
Season (SP)	0.003458	0.067826	0.051	0.959
Season (SU)	0.003095	0.065648	0.047	0.962
Season (WI)	-0.12529	0.061703	-2.031	**0.042**
Temperature (LT)	-0.39047	0.091326	-4.276	**0.008**
Temperature(MT)	-0.18453	0.051122	-3.61	**0.003**

(Significance <0.05)

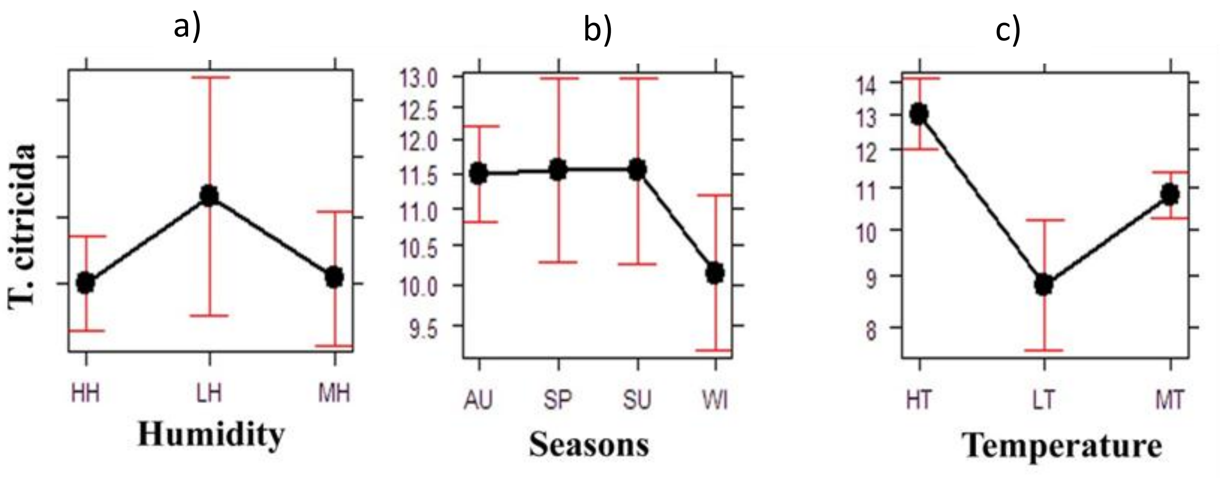

Fig 7 : Effect of different variables on abundance of *T.citricida*. a) effect of relative humidity on abundance of *T.citricida* b) effect of seasons on abundance of *T.citricida* d) effect of temperature on abundance of *T.citricida*.

4.4 Effects of physical parameters on *Toxoptera aurantii*

T. aurantii also recorded as vectors for the transmission of CTV in citrus from Kavrepalanchok. The abundance *of T. aurantii* was affected by various physical parameters. Population of *T. aurantii* was changed with the changes in temperature, humidity and seasons. During this study the population of *T.aurantii* was found significantly affected by moderate and lower temperature ($p<0.05$). Population of *T. aurantii* was recorded high when the temperature was in the range of 21^0C- 24^0C but least population was found when temperature was below 20^0C (fig-8, c). Like temperature humidity also found a key factor to affect the population of *T. aurantii*. During this study period the population was found significantly affected by lower humidity ($p<0.05$). The population abundance of *T. aurantii* was found to be maximum during the period when humidity was low and minimum population was trapped in high humid air (Fig 8 a). This study showed the population of *T. aurantii* was significantly affected by winter season ($P<0.05$). Population abundance of *T. aurantii* was affected by change in seasons. In this study the abundance of *T. aurantii* was found to be significantly affected by winter season ($P<0.05$) (Table 4). During this study period the population of *T. aurantii* was found low in winter seasons and high in summer season (Fig 8 b)

Table 4 Result obtained from GLM of *T. aurantii* with variables (temperature, relative humidity and seasons) (GLM formula = *T. aurantii* ~ relative humidity + seasons + temperature, family = poisson(log)

Variables	**Estimate**	**Standard Error**	**Z-test**	**P- value**
(Intercept)	0.3636	0.1755	2.072	**0.038**
Humidity (LH)	0.6547	0.2911	2.249	**0.024**
Humidity (MH)	0.2927	0.1912	1.531	0.125
Season (SP)	0.29	0.2136	1.358	0.174
Season (SU)	-0.2818	0.2406	-1.171	0.241
Season (WI)	0.3901	0.1845	2.115	**0.034**
Temperature(LT)	-0.8918	0.3436	-2.596	**0.009**
Temperature (MT)	-0.6347	0.1565	-4.056	**0.001**

(Significance <0.05)

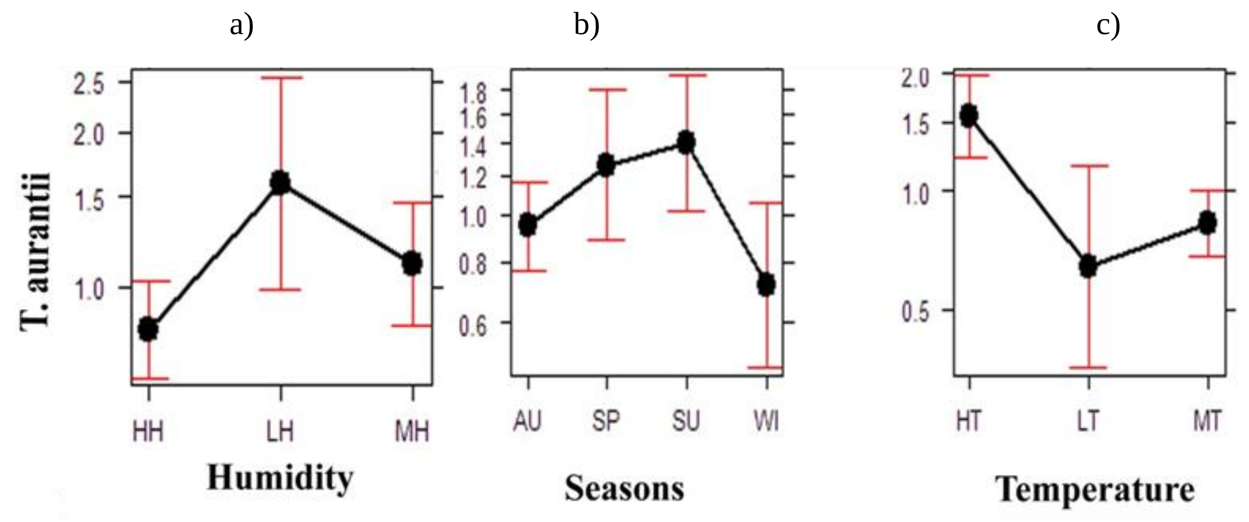

Fig 8 : Effect of different variables on abundance of *T. aurantii* a) effect of humidity on abundance of *T. aurantii* b) effect of seasons on abundance of *T. aurantii* c) effect of temperature on abundance of *T.aurantii*

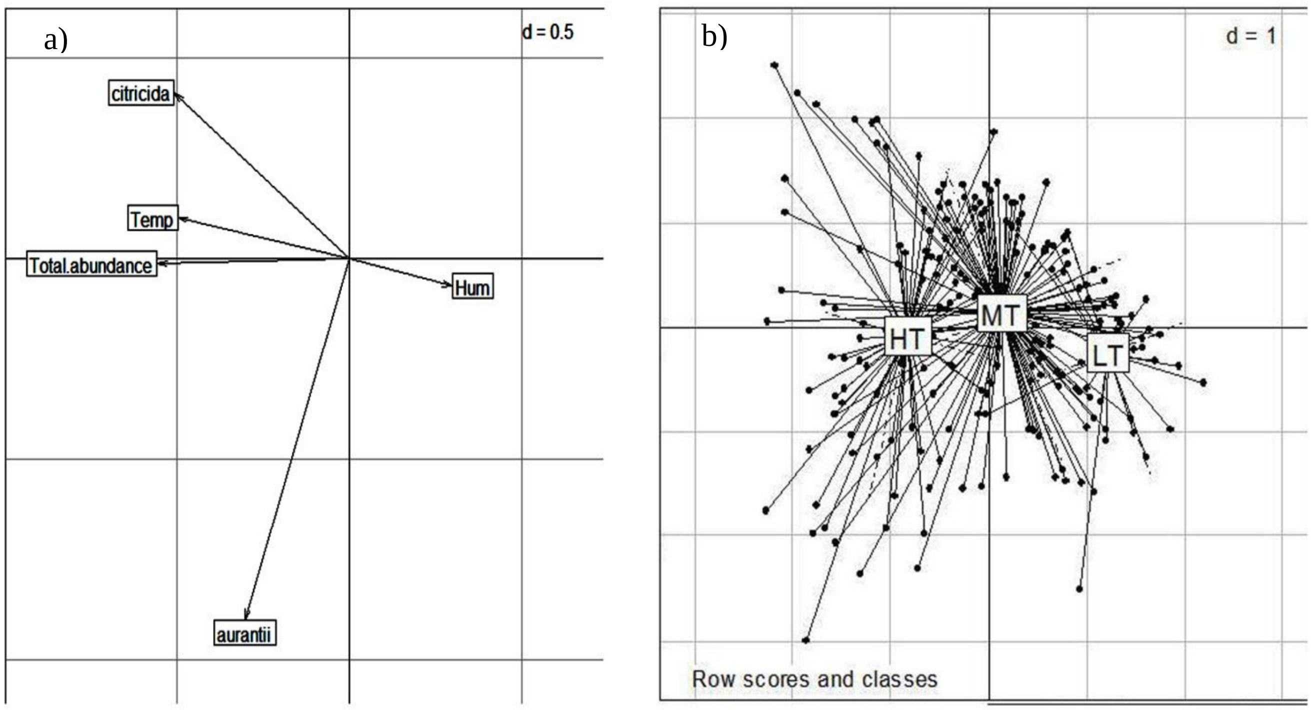

Fig 9 : Multivariate Analysis a) PCA of total abundance of aphids and abundance of identified species (*T. citricida* and *T. aurantii*) with temperature and relative humidity b) DA of abundance data of aphids with respect to temperature.

Total abundance of aphids were found followed with temperation (Temp) and negative relation with humidiy (Hum). The abundance of *T. citricida* was found very high than *T. aurantii*. Maximum abundance of aphid was found in moderate temperature (MT) and minimum in lower temperature (LT), this means the moderate temperature (MT) significantly affect the abundance of CTV vectors.

4.5 Effects of Aspects on CTV vectors

Abundance of CTV vectors were affected with different aspects. Total abundance of aphid and *T. citricida* were higher in western aspect and lower in northern aspects. But in case of *T. aurantii*, abundace was found higher in northern aspects and lower in western aspect. This might be different climatic parameters and ecology of different species of aphids. Statistically there was no significant effect on CTV vectors by aspects (P> 0.05). This might be different climatic parameters and ecology of different species of aphids.

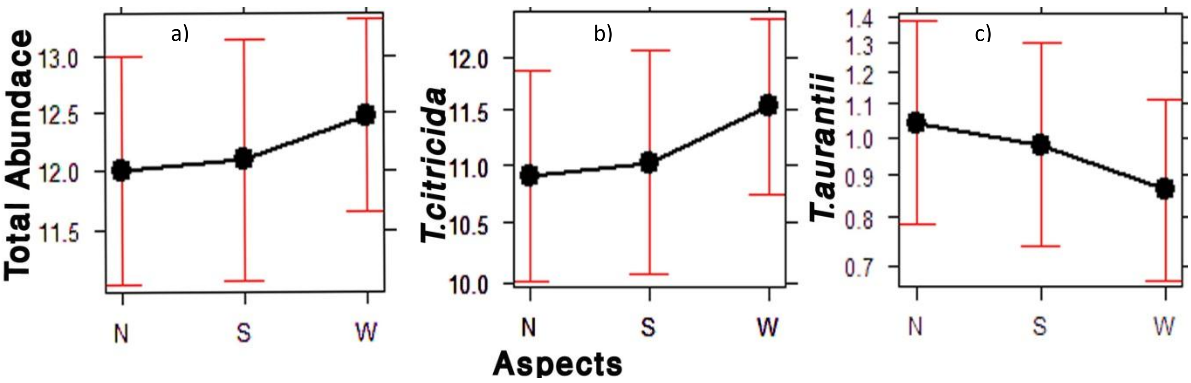

Fig: 10 Effects of aspects on CTV vectors a) Effects of aspects on total abundance of vectors b) Effects of aspects on *T.citricida* c) Effects of aspects on *T. aurantii*

4.6 Month-wise CTV symptoms (Stem pitting and Vein clearing)

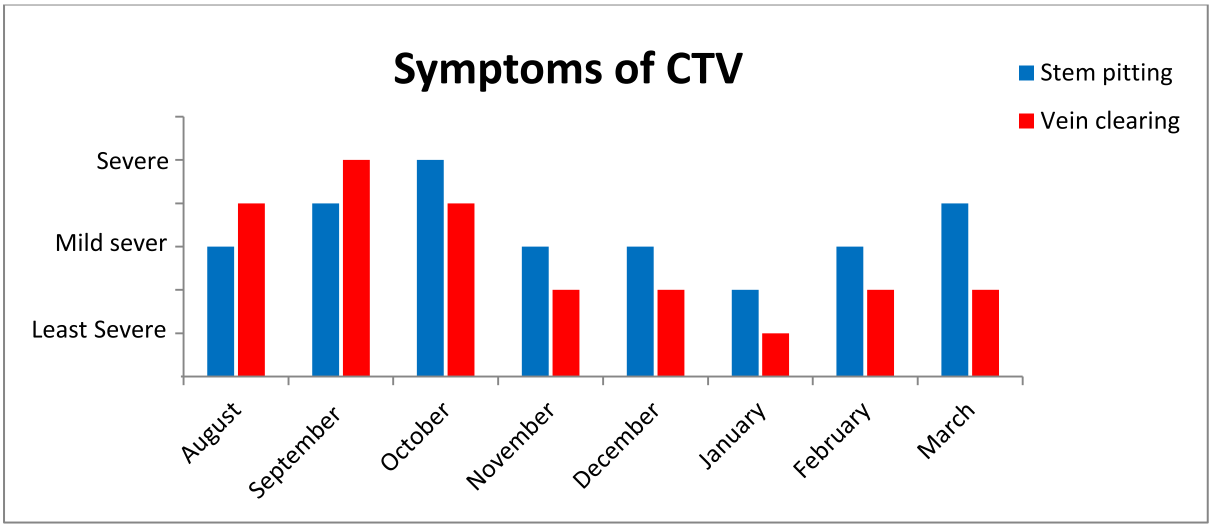

Fig 11: Month-wise CTV symptoms (Stem pitting and Vein clearing)

Stem pitting and vein clearing are major physical symptoms of citrus tristeza virus. In this study, severe vein clearing and stem pitting was recorded in the month of September and October respectively. While least severe of CTV symptoms were found to be in month of January. In month August and September, CTV symptom vein clearing was found higher in comparing to stem pitting, besides these two months stem pitting symptoms was found to be dominant.

5 DISCUSSION

In this study two species of aphids namely *T. citricida* and *T.aurantii* were found from citrus farm Kavrepalanchowk as a vector of CTV, *T. citricida* was found as a key species for the transmission of CTV as the population abundance of *T. citricida* were more than 90 % of total collected aphids. Similarly, Regmi and Lama (1992) reported two species of aphid *T. citricida* and *T. aurantii* from the horticulture farm of Pokhara and Sanothimi (Bhaktapur) among which *T. citricida* was considered as a major vector for the transmission of the CTV. Likewise, similar species of aphids were recorded for the transmission of CTV in horticulture farms at Lamjung, Nepal (Ghimire, 2000). In all citrus growing countries CTV is the most economically important virus pathogens. Citrus vectors are sensitive to the climatic factors; different vectors are responsible for transmission of CTV in different part of world (Rosistacher, 1991). *A. gossypii, T. citricida* and *A. spiraecola* were recorded from citrus orchard; *T. citricida* comprises 88% of total aphid species and concluded a main vector for the transmission of CTV in mandarin farm of South East Asia (Bayhan *et al.*, 2006). Except these two aphids vectors other vectors also were found for the transmission of CTV vectors in different countries of world. Yumruktepe (1993) carried out an aphid survey in the eastern Mediterranean Region. *Aphis citricola, A. Gossypii, A. craccivora, T. aurantii* and *Myzus persicae* were detected in the region. *A. citricola* and *A. gossypii* were found to be the major aphid species among the others. This result differ with the result of this study, this difference might be absence of key vector *T. citricida* in eastern Mediterranean Region.

Temperature and humidity were found to be the major factors for population fluctuation of citrus aphid. In this study, peak population was reported when the temperature was 25^0C and population was found least when temperature was below 20^0C. Peak infestation and number of auxiliaries were observed mainly in spring in autumn, and in summer when the temperature was more than 24^0C. This temperature was found favorable for production of new shoots in citrus tree that was appropriate for the reproduction of citrus aphids. Similarly, Acromonte (1994) recorded that the abundance of CTV vectors increases with increase in temperature of the environment. Likewise in this study, only 156 aphid specimens were collected at 15^0 C during the month of January and study the abundance of aphids were found to be increased up to 80/trap (average) when temperature of the farm rose to 32^0 C during the month of March. In the previous study also the peak of infestation of citrus aphids on citrus tree was recorded in spring and in autumn (Saharaoui and Hemptinne, 2009; Kamel, 2010; Yoldas *et al*., 2011; Mostefaoui *et al*., 2014). In the previous research high population of the aphids was recorded in spring season when temperature was 36^0 C and least in winter (Michaud and Browining, 1999). Nickel and Klingaut (1985) studied the *T. citricida* population dynamics for two years in Argentina. Periods of heavy rain and hot dry weather were both correlated low *T.citricida* population density that might be due to fluctuation in temperature and humidity. Aderson (1914) also noted the citrus aphid population was checked by heavy showers that might be decrease in temperature. In the present study, 285 aphids were trapped in the month of July but aphid populations was found gradually decreased in same month in third week of July that might be due to decrease in temperature and increase in humidity due to rainfall.

During this study period high abundance of aphid population was observed in average humidity of 71% during the month of summer and lowest population in average humidity of 51% during winter on the first week of January. In the same way, Komazaki (1982) reported that the intrinsic rate of increase for aphid population was recorded highest when humidity was 77%. Nickle and Klingaut (1985) reported minimum diversity and abundance of citrus aphids due to extreme humidity in winter. Tsai and wang (1999) also observed the optimal range of humidity (60- 80%) for *T. citricida*. Previous study recorded number of alate aphids was determined humidity in the area (Sharma, 1968). In the study carried out by Jothi *et al.* (1998) recorded the abundance of *T. aurantii* fluctuation was due to many factors, like temperature, humidity, seasons, and rainfall. He recorded lower temperature, higher humidity and a winter season limits the population of *Toxoptera aurantii*. Similarly, population abundance of aphids was checked by lower temperature, higher humidity and winter season in Kavrepalanchok, the greatest numbers of citrus aphids were associated with lower humidity (mid-February to mid-April).

Population of citrus aphids was recorded highest in citrus farm of Kavrepalanchok during the month of June and lowest in the month of January similarly previous result recorded high number of aphid colonies in summer season than in winter (Panday *et al.*, 1990). Kinlmann and Dixon (2010) reported dynamics of aphids were largely determined by seasonal changes in host quality. Aphids do best when amino acids are actively translocated in the phloem. Thus on trees, the leaves found to be most suitable for aphids in springs and autumn.

Many author mentioned the importance of seasons for the biology of aphids (Bayhan *et al*., 2006; Dixon and Hopkins, 2010; Harrington and Clark, 2010; Geo *et al*., 2013). During this study period the fluctuations of aphid population and infestation level seem to be influenced by the changes of seasons. High aphid specimens were collected when temperature was more than 25^0C during summer season. The study carried out in different citrus orchard also suggested that citrus aphids were most abundant during late August to early November (Pandey, *et al*., 1986; Kandoria *et al*., 1989; Kohilla *et al*., 1996). Kfir and Rosen (1981) studied the biology of the aphids on lemon farm of Brazil and least diversity and abundances of *T.citricida* was recorded during autumn seasons. In contrast, present study showed number of *T. citricida* was low during winter seasons.

The symptoms of stem pitting and vein clearing were observed in all citrus trees of Kavrepalanchok farm. It clearly indicated that there was a strain of CTV which cause symptoms of stem pitting and vein clearing in citrus farm Kavrepalanchok. Similar CTV strain was reported from different citrus farm of Nepal (Regmi *et al*., 2000). Similar observations were also reported from Ramechhap and Sindhuli districts (Tomyasu and

Verma, 1990). Pandey *et al*. (1990) observed CTV a symptom during the month of September similarly a symptom of CTV was recorded very severe during the month of September and October in the citrus farm of Kavrepalanchok. The study carried in the citrus farm of Greece showed high stem pitting symptoms in the month of January (Dimou *et al*., 2001). This result contradicted with the result of this study that might be due to difference in climatic condition and species of aphids involve for the transmission of the CTV.

6 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Two species of aphid vectors namely *T. citricida* and *T. aurantii* are reported, among which *T. citricida* is found to be key vector in Kavrepalanchowk, Nepal. Population abundances of aphids are found to be affected by climatic parameters. The population abundance of aphids is found to be increased with increase in temperature and decrease in humidity. Similarly, the population abundance of aphids are found to be highest in summer season and lowest in winter season. CTV symptoms, stem pitting is found to be severe in the month of October and vein clearing is found to be highest in the month of September and both CTV symptoms are found to be least in the month of January.

6.2 Recommendations

- For the sustainable management of CTV vectors detail taxonomic and ecological study should be carried in present study area.
- Similar kinds of study should be replicated in other citrus growing areas of Nepal, for sustainable management of vectors and to promote the production of mandarin.

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APPENDICES

Collected Data

Code	Aspect	month	Season	Hum1	temp1	Hum	Temp	*citricida*	*aurantii*	Total abundance
ND2T2	N	D	AU	HH	LT	82	20	8	0	8
SD2T3	S	D	AU	MH	LT	52	20	7	2	9
SD3T3	S	D	AU	MH	MT	54	21	9	0	9
SD3T2	S	D	AU	MH	MT	56	22	6	3	9
WD1T3	W	D	AU	HH	MT	63	22	9	0	9
WD3T1	W	D	AU	HH	MT	69	22	9	0	9
ND3T3	N	D	AU	HH	LT	78	20	8	1	9
ND2T1	N	D	AU	HH	LT	79	19	8	1	9
ND3T1	N	D	AU	HH	LT	80	20	9	0	9
SD1T2	S	D	AU	MH	MT	54	22	10	0	10
SD2T1	S	D	AU	MH	MT	54	22	10	0	10
SD1T3	S	D	AU	MH	MT	57	22	10	0	10
WD3T3	W	D	AU	HH	MT	66	22	9	1	10
WD2T3	W	D	AU	HH	MT	67	22	10	0	10
ND1T1	N	D	AU	HH	MT	75	22	10	0	10
ND1T3	N	D	AU	HH	MT	77	21	10	0	10
SD2T2	S	D	AU	MH	MT	56	22	11	0	11
WD3T2	W	D	AU	HH	MT	64	23	10	1	11
WD2T1	W	D	AU	HH	MT	64	24	11	0	11
ND3T2	N	D	AU	HH	LT	79	20	10	1	11
ND2T3	N	D	AU	HH	MT	81	21	10	1	11
SD1T1	S	D	AU	MH	MT	56	21	12	0	12
WD1T1	W	D	AU	HH	MT	64	24	12	0	12
ND1T2	N	D	AU	HH	MT	74	22	12	0	12
SD3T1	S	D	AU	MH	MT	58	21	11	2	13
WD1T2	W	D	AU	HH	HT	62	25	13	0	13
WD2T2	W	D	AU	HH	HT	64	25	13	1	14
NN1T1	N	N	AU	HH	MT	69	24	11	0	11
WN1T1	W	N	AU	HH	MT	61	24	12	0	12

NO3T1	N	N	AU	HH	MT	70	23	11	1	12
NN1T2	N	N	AU	HH	MT	72	23	11	1	12
SN1T3	S	N	AU	LH	MT	42	23	11	2	13
NN3T1	N	N	AU	HH	MT	69	24	13	0	13
NN3T2	N	N	AU	HH	MT	70	23	13	0	13
SN1T2	S	N	AU	LH	MT	39	23	12	2	14
SN3T3	S	N	AU	LH	MT	47	23	14	0	14
WN2T1	W	N	AU	MH	MT	59	24	14	0	14
WN3T3	W	N	AU	HH	HT	65	25	11	3	14
NN2T2	N	N	AU	HH	HT	66	25	14	0	14
NN1T3	N	N	AU	HH	MT	68	24	11	3	14
SN3T2	S	N	AU	LH	MT	45	22	12	3	15
SN2T3	S	N	AU	LH	MT	46	22	13	2	15
SN2T2	S	N	AU	LH	MT	48	23	11	4	15
WN1T3	W	N	AU	MH	HT	59	27	15	0	15
WN3T1	W	N	AU	MH	HT	60	25	14	1	15
NN2T1	N	N	AU	HH	HT	65	25	14	1	15
SN2T1	S	N	AU	LH	MT	41	24	16	0	16
WN2T3	W	N	AU	MH	HT	54	26	13	3	16
NN2T3	N	N	AU	HH	MT	67	24	13	3	16
SN1T1	S	N	AU	LH	HT	41	26	17	0	17
SN3T1	S	N	AU	LH	MT	42	23	15	2	17
WN1T2	W	N	AU	HH	HT	62	27	16	1	17
NN3T3	N	N	AU	HH	MT	69	24	13	4	17
WN2T2	W	N	AU	MH	HT	60	25	16	4	20
WN3T2	W	N	AU	HH	HT	68	28	20	2	22
NO3T3	N	O	AU	HH	MT	69	24	10	0	10
SO1T3	S	O	AU	LH	MT	45	21	7	4	11
NO2T3	N	O	AU	HH	MT	69	23	10	1	11
NO1T1	N	O	AU	HH	MT	70	23	9	2	11
NO3T2	N	O	AU	HH	MT	71	23	11	0	11

SO2T3	S	O	AU	LH	MT	48	22	9	3	12
NO1T3	N	O	AU	HH	MT	69	24	12	0	12
SO3T2	S	O	AU	LH	MT	45	23	13	0	13
WO2T3	W	O	AU	HH	MT	61	24	13	0	13
NO2T1	N	O	AU	HH	MT	68	24	12	1	13
SO1T2	S	O	AU	LH	MT	44	23	14	0	14
SO3T3	S	O	AU	LH	MT	47	24	14	0	14
WO3T3	W	O	AU	MH	HT	59	25	14	0	14
WO1T1	W	O	AU	MH	HT	60	25	13	1	14
NO2T2	N	O	AU	HH	HT	66	25	14	0	14
NO1T2	N	O	AU	HH	MT	67	24	13	1	14
SO2T2	S	O	AU	LH	MT	41	23	15	0	15
SO1T1	S	O	AU	LH	HT	47	25	12	3	15
WO3T2	W	O	AU	MH	HT	60	25	11	4	15
WO1T3	W	O	AU	HH	MT	62	24	14	1	15
WO2T1	W	O	AU	HH	MT	63	24	13	2	15
SO2T1	S	O	AU	LH	HT	42	25	15	1	16
WO1T2	W	O	AU	MH	HT	58	27	15	2	17
WO2T2	W	O	AU	MH	HT	60	26	18	2	20
SO3T1	S	O	AU	LH	MT	43	24	16	1	17
WO3T1	W	O	AU	HH	MT	62	24	13	0	13
SS3T1	S	S	AU	LH	MT	42	21	7	1	8
SS2T1	S	S	AU	MH	MT	56	22	8	0	8
NS3T1	N	S	AU	HH	MT	74	22	9	0	9
WS2T1	W	S	AU	MH	HT	58	26	10	0	10
SS2T3	S	S	AU	LH	MT	49	22	10	1	11
SS1T1	S	S	AU	MH	MT	53	24	10	1	11
WS3T1	W	S	AU	HH	MT	61	24	11	0	11
WS3T3	W	S	AU	HH	MT	62	24	11	0	11
NS1T1	N	S	AU	HH	MT	72	24	10	1	11
SS1T3	S	S	AU	MH	HT	54	25	11	1	12

WS1T1	W	S	AU	MH	HT	54	26	11	1	12
NS1T2	N	S	AU	HH	HT	68	26	11	1	12
NS3T3	N	S	AU	HH	MT	70	23	11	1	12
SS1T2	S	S	AU	MH	HT	58	25	12	1	13
WS2T3	W	S	AU	MH	MT	59	24	13	0	13
NS3T2	N	S	AU	HH	MT	69	24	13	0	13
NS1T3	N	S	AU	HH	HT	69	25	11	2	13
NS2T1	N	S	AU	HH	HT	71	25	12	1	13
SS3T2	S	S	AU	LH	MT	46	24	12	2	14
SS3T3	S	S	AU	LH	MT	48	24	11	3	14
NS2T2	N	S	AU	HH	HT	66	26	12	2	14
SS2T2	S	S	AU	MH	MT	51	23	11	4	15
WS1T3	W	S	AU	MH	HT	52	25	15	0	15
WS1T2	W	S	AU	MH	HT	55	27	13	3	16
NS2T3	N	S	AU	HH	HT	61	28	13	3	16
WS3T2	W	S	AU	HH	HT	61	26	16	3	19
WS2T2	W	S	AU	MH	HT	54	25	18	4	22
NM2T2	N	M	SP	HH	MT	71	22	9	0	9
NM3T1	N	M	SP	HH	MT	68	23	10	0	10
WM3T1	W	M	SP	HH	MT	70	21	10	0	10
NM1T2	N	M	SP	HH	MT	74	21	8	2	10
WM3T3	W	M	SP	HH	LT	75	20	7	3	10
WM2T3	W	M	SP	HH	MT	67	23	11	0	11
NM3T2	N	M	SP	HH	MT	72	22	10	1	11
NM1T1	N	M	SP	HH	MT	76	22	10	1	11
SM1T1	S	M	SP	HH	MT	61	24	11	1	12
WM2T1	W	M	SP	HH	MT	64	24	12	0	12
WM1T3	W	M	SP	HH	MT	65	24	12	0	12
NM1T3	N	M	SP	HH	MT	69	22	11	1	12
NM3T3	N	M	SP	HH	MT	71	22	11	1	12
SM2T1	S	M	SP	HH	HT	62	25	9	4	13

WM2T2	W	M	SP	HH	HT	63	25	13	0	13
WM1T1	W	M	SP	HH	HT	68	25	11	2	13
NM2T1	N	M	SP	HH	MT	72	22	12	1	13
SM2T2	S	M	SP	MH	HT	59	26	13	1	14
NM2T3	N	M	SP	HH	MT	69	24	12	2	14
WM1T2	W	M	SP	HH	HT	70	25	13	1	14
WM3T2	W	M	SP	HH	MT	71	21	12	2	14
SM1T2	S	M	SP	MH	HT	59	25	12	3	15
SM3T2	S	M	SP	HH	MT	61	24	15	0	15
SM3T1	S	M	SP	HH	MT	63	24	15	0	15
SM3T3	S	M	SP	MH	HT	59	25	16	2	18
SM1T3	S	M	SP	MH	HT	60	27	13	6	19
SM2T3	S	M	SP	HH	HT	63	29	19	1	20
NA1T2	N	A	SU	HH	MT	74	23	9	1	10
SA1T2	S	A	SU	HH	MT	62	22	10	1	11
NA1T1	N	A	SU	HH	HT	68	27	9	2	11
WA2T1	W	A	SU	MH	MT	58	24	11	1	12
SA3T1	S	A	SU	MH	MT	59	24	11	1	12
NA3T2	N	A	SU	HH	MT	74	24	10	2	12
WA2T3	W	A	SU	MH	HT	60	26	11	2	13
SA1T1	S	A	SU	HH	MT	64	21	12	1	13
NA3T1	N	A	SU	HH	HT	71	25	12	1	13
SA3T2	S	A	SU	MH	HT	54	25	12	2	14
WA1T1	W	A	SU	MH	MT	57	24	14	0	14
SA2T1	S	A	SU	HH	HT	61	26	11	3	14
NA2T2	N	A	SU	HH	HT	68	26	13	1	14
WA3T3	W	A	SU	MH	HT	57	26	14	1	15
WA2T2	W	A	SU	MH	HT	59	25	12	3	15
SA1T3	S	A	SU	HH	HT	63	27	11	4	15
NA2T1	N	A	SU	HH	HT	68	27	14	1	15
SA3T3	S	A	SU	MH	HT	52	25	13	3	16

WA1T2	W	A	SU	MH	HT	54	25	14	2	16
SA2T2	S	A	SU	HH	HT	61	27	15	1	16
WA3T1	W	A	SU	HH	HT	62	26	14	2	16
NA2T3	N	A	SU	HH	HT	67	26	13	3	16
WA1T3	W	A	SU	MH	MT	56	24	14	3	17
NA3T3	N	A	SU	HH	HT	69	27	15	2	17
NA1T3	N	A	SU	HH	HT	67	27	16	2	18
SA2T3	S	A	SU	MH	HT	60	28	16	3	19
WA3T2	W	A	SU	MH	HT	59	26	16	4	20
WF1T1	W	F	WI	HH	LT	69	20	7	0	7
NF1T1	N	F	WI	HH	LT	78	20	6	1	7
SF1T3	S	F	WI	MH	LT	57	20	8	0	8
NF2T2	N	F	WI	HH	LT	74	20	8	0	8
SF3T2	S	F	WI	MH	MT	60	21	7	2	9
SF3T3	S	F	WI	HH	MT	62	21	7	2	9
WF1T3	W	F	WI	HH	LT	67	20	8	1	9
WF2T1	W	F	WI	HH	LT	70	20	8	1	9
WF3T1	W	F	WI	HH	MT	71	21	9	0	9
NF3T3	N	F	WI	HH	MT	72	21	9	0	9
NF2T1	N	F	WI	HH	LT	77	20	9	0	9
NF3T1	N	F	WI	HH	MT	78	22	9	0	9
SF1T2	S	F	WI	MH	MT	59	23	8	2	10
SF2T3	S	F	WI	HH	LT	61	20	10	0	10
SF2T1	S	F	WI	HH	MT	61	21	10	0	10
WF3T3	W	F	WI	HH	MT	68	21	10	0	10
WF2T2	W	F	WI	HH	MT	70	21	10	0	10
WF2T3	W	F	WI	HH	MT	72	21	10	0	10
NF1T2	N	F	WI	HH	MT	77	21	8	2	10
SF3T1	S	F	WI	MH	MT	59	23	10	1	11
SF2T2	S	F	WI	HH	MT	62	23	11	0	11
WF1T2	W	F	WI	HH	MT	68	23	10	1	11

WF3T2	W	F	WI	HH	MT	69	24	10	1	11
NF3T2	N	F	WI	HH	MT	72	21	9	2	11
NF2T3	N	F	WI	HH	MT	75	22	10	1	11
SF1T1	S	F	WI	MH	MT	57	24	11	1	12
NF1T3	N	F	WI	HH	MT	79	22	10	2	12
WJ1T1	W	J	WI	HH	LT	69	20	7	0	7
NJ1T1	N	J	WI	HH	LT	82	19	7	0	7
SJ1T3	S	J	WI	MH	LT	51	20	7	1	8
NJ2T2	N	J	WI	HH	MT	79	21	8	0	8
SJ3T2	S	J	WI	MH	LT	58	20	9	0	9
SJ3T3	S	J	WI	MH	LT	58	20	9	0	9
WJ1T3	W	J	WI	HH	LT	71	20	9	0	9
WJ2T1	W	J	WI	HH	LT	72	20	9	0	9
WJ3T1	W	J	WI	HH	MT	72	21	9	0	9
NJ2T1	N	J	WI	HH	MT	79	21	8	1	9
NJ3T3	N	J	WI	HH	MT	79	21	9	0	9
NJ3T1	N	J	WI	HH	LT	80	20	9	0	9
SJ1T2	S	J	WI	MH	MT	52	21	10	0	10
SJ2T1	S	J	WI	MH	MT	52	21	10	0	10
SJ2T3	S	J	WI	MH	LT	54	20	10	0	10
WJ3T3	W	J	WI	HH	MT	70	21	8	2	10
WJ2T3	W	J	WI	HH	MT	71	22	10	0	10
WJ2T2	W	J	WI	HH	MT	73	21	9	1	10
NJ1T2	N	J	WI	HH	MT	81	21	10	0	10
SJ2T2	S	J	WI	MH	MT	54	21	11	0	11
SJ3T1	S	J	WI	MH	MT	56	23	11	0	11
WJ1T2	W	J	WI	HH	MT	70	21	10	1	11
WJ3T2	W	J	WI	HH	MT	71	22	10	1	11
NJ2T3	N	J	WI	HH	MT	78	21	10	1	11
NJ3T2	N	J	WI	HH	MT	78	22	11	0	11
SJ1T1	S	J	WI	MH	MT	55	23	12	0	12

NJ1T3	N	J	WI	HH	MT	80	21	11	1	12

Photographs During field and lab work

Photograph 1 Collecting Aphids trapped on Yellow Trap

Photograph 2 Preparations of Permanent Slides

Photograph 3 Installing yellow traps

Photograph 4 Citrus farm of Kavrepalanchowk district