LADYBIRD BEETLES AND THEIR ASSOCIATION WITH APHID WITHIN TRIBHUVAN UNIVERSITY PREMISES KIRTIPUR, NEPAL

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Submitted to

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DECLARATION

I hereby declare that the work presented in this thesis entitled "Ladybird Beetles and their Association with Aphid within Tribhuvan University Premises, Kirtipur, Nepal" has not been published or submitted elsewhere for the requirement of degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

Date: ..09/03/2021

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On the recommendation of supervisor "Dr. Prem Bahadur Budha" this thesis submitted by Sushila Bajracharya entitled "Ladybird Beetles and their Association with Aphid within Tribhuvan University Premises, Kirtipur, Nepal" is approved for the examination in partial fulfilment of the requirements for Master's Degree of Science in Zoology with special paper Entomology.

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Sushila Bajracharya entitled "Ladybird Beetles and their Association with Aphid within Tribhuvan University Premises, Kirtipur, 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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ABSTRACT

Ladybird beetles (Coleoptera: Coccinellidae) are economically important predators of pests species viz. aphids. This research aimed to explore species diversity of ladybird beetles, found association with their aphid prey species and temperature in Tribhuvan University area premises, Kirtipur. It also compared feeding efficiency among two selected coccinellid predators. Visual observation was done for ladybird beetles and aphid prey species from 14 May to 9 November, 2019 in summer, rainy and autumn seasons, collected and identified. A total of 17 species of lady bird beetles belonging to 12 genera and four tribes (Coccinellini, Epilachni, Sticholotini and Noviini) under the subfamily Coccinellinae of order Coleoptera were reported. The Shannon Diversity index (H) was 1.25 with *C. septempunctata* (71.78%) as the most dominant species. Seasonally, the diversity was highest in autumn (H=1.51) as compared to rainy (H= 0.96) and summer (H= 0.85). The diversity was highest in agricultural land (H=1.44, J=0.56) followed by garden (H=1.36, J=0.69), planted forest (H=1.25, J=0.70) and grassland (H=0.96, J=0.38). The abundance of ladybird beetles showed significant positive relation with temperature and summer season. The prey aphids associated with beetle fauna were *Macrosiphoniella pseudoartemisiae*, *M. yomogifoliae*, *Rhopalosiphum maidis*, *Aphis gossypii* and *Macrosiphum rosae*. The feeding efficiency experiment conducted in room temperature, the efficient predator of *Lipaphis erysimi* was found *C. septempunctata* (34.40 ± 1.67) than *P. luteopustulata* (29.15 ± 1.84). Since ladybird diversity is high in T.U., especially highly abundant *C. septempunctata*, it can be used for biological control of mustard aphid pests.

1. INTRODUCTION

1.1 Background of the study

Ladybird beetles belong to the family Coccinellidae of the order Coleoptera. There are about 6000 species and 360 genera of ladybird beetles distributed worldwide (Vandenberg 2000, Slipinski 2007) including some geographically restricted species (Majerus and Kern 1989). They are reported in tundra, forest, grassland and agroecosystems (Iperti 1999). There are 235 nominal species of ladybird beetles reported from Nepal belonging to 57 genera and six subfamilies including 26 endemic species (Thapa 2015). Coccinellidae is divided into two subfamilies, Microweiseinae and Coccinellinae, and twenty four tribes (Slipinski 2007, Giorgi et al. 2009, Seago et al. 2011, Robertson et al. 2015).

The abundance and the diversity of coccinellids influence the population of pest and prey in agricultural crop land (Obrycki and Kring 1998, Altieri 1999). Adult coccinellids emerge in the spring and disperse, then enter aestivation and hibernation in summer and autumn respectively depending on species (Iperti 1999). They are generalist among the predaceous beetle groups (Giorgi et al. 2009). Coccinellid activities are comparatively higher in small landscapes with a variety of crops along with semi-natural habitats (viz. grasslands and forests) than in the large landscapes dominated by annual crops only (Woltz and Landis 2014). Urban agroecosystems also change abundance and species richness of beetles in any positive or negative pattern according to change in urbanization history (Egerer et al. 2018). Coccinellid community composition, species diversity, species richness and dominance significantly changes during each vegetative pattern (Honek et al. 2015). Agriculture, habitat changes, invasive species and climate changes influence coccinellid species in different ways (Honek et al. 2017).

Coccinellids are economically important insects due to their predatory nature against pest species of agricultural crops (Rafi et al. 2005) however some species are pest as well. During disturbances in crop lands, these natural enemies get food resources and shelter from semi- natural habitats, but spatial arrangement of habitats within a landscape can affect the arrival into crop fields (Bianchi et al. 2006, Woltz and Landis 2014). So, weeds present in the borders of field crops can help in biological conservation of natural enemies (Wackers and van Rijn 2012, Shanker et al. 2018).

Both larvae and adult ladybird beetles feed on various insect pests of agricultural crops and fruit plants such as aphids, mealybugs, whiteflies, thrips, jassids, and psyllids (Ullah et al. 2012). Ladybird beetles also feed on diversified food items such as myrmecophilous (Majerus et al. 2006), acariphagous (Biddinger et al. 2009), aphidophagous mycophagous, and polyphagous (Sutherland and Parrella 2009) and some are phytophagous or pollinivores (Genus *Bulaea, Coleomegilla* and *Micraspis*) (Giorgi et al. 2009). Ladybird beetles vary in sizes ranging from minute to large (0.8 – 28 mm) in length and have usually oval or rounded bodies with distinctly convex dorsal body parts (Slipinski and Tomaszewska 2010). Elytral colour varies different trophic range from bright red, yellow, pink in aphidophagous coccinellid, dark in coccidophagous coccinellid to light maroon, white or lemon yellow in mycophagous coccinellid (Iperti 1999). The morphs of ladybird beetle species differ in the number and location of the spots and colour on their elytra due to which they are widely used in determining local and temporal trends (Honek et al. 2012). The melanic morphs increase with increased cold and humid conditions to adapt the environmental condition (Dobzhansky 1933).

Coccinellid can be used as bioindicator insects since they are very vulnerable to environmental changes, including events that influence the natural enemies of coccinellids, i.e., predators, parasitoids and pathogens, and are sensitive to the effects of physical and chemical pollution, including microclimatic fluctuations (Iperti 1999). Various natural enemies viz., bacteria, fungi, mites, nematodes, protozoa, wasps, flies (Riddick et al. 2009), spiders, fish, amphibian, reptiles, aves, and mammal are present although being distasteful and toxic reflex bleeding (Ceryngier et al. 2012). Their blood (hemolymph) is yellow, has a disgusting smell and may contain antigenic poison in some species which is regarded as a way of keeping away predators and making ladybirds a secure species (Albaaj 2017). The effect of temperature on survival, fecundity, demographic parameters, growth rate and development, which is used for predicting interaction among bio control agents and their host insects (Lemoine and Burkepile 2012). In coccinellids, as in other exotherms, life span shortens with increasing temperature. The degree of their adaptation as well as their efficiency in controlling aphid populations observed varies with the species and the environmental conditions (Dixon 2000), nutritional status (Thompson 1999), suitability of the prey for the growth and reproduction of the predator (Hodek and Honek 1996), prey size (Isikber 2005) and effect of host plant of prey (Wu et al. 2010).

Due to their predaceous nature, ladybird beetles have been used as biological control agents of agricultural insect pests, particularly aphids. Insect pests are major limiting factors to crop production systems, which cause about 12-15% crop losses worldwide (Upadhyaya 2003) and 15- 20% in Nepal (Joshi et al. 1991, Palikhe et al. 2003, Khanal et al. 2014). Aphids are among the serious pests of many crops in Nepal. There are 22 species of aphids which are pests of fruits (apple, banana, citrus, plum), vegetables (pea, cowpea, cabbage, potato) cereals (maize, rice, wheat) and other cash crops (cotton mustard) (http://dftqc.gov.np).

The association between ladybirds and aphids was recognized centuries ago (Omkar and Pervez 2016). Prey-predator interactions are spatially and temporally dynamic (Park and Obrycki 2004). Aphids are the largest phytophagous insect community causing a great economic loss to agricultural crops (Prabhakar and Roy 2010). The aphid population density is regulated by density-dependent processes (Sequeira and Dixon 1997). Aphidophagous predators occur in distinct patches (colonies). Therefore, food availability is unlikely to be the main limiting factor for adult predators (Kindlmann and Dixon 2001).

Several attempts have been made in the world to control aphids by using predaceous ladybird beetles (Joshi et al. 2003, Khan et al. 2009).The seven-spotted ladybird *Coccinella septempunctata* is a widely distributed predator that feeds on about 106 prey species. It is a natural predator of the mustard aphid pest *Lipaphis erysimi* in tropical regions (Omkar and Pervez 2002). The prey range of *Propylea* is small due to its small size (Pervez and Omkar 2011). Ladybird beetle species *C. septempunctata* has been used as the biological control of aphids in the world (Shands et al. 1966, Stiling and Cornelissen 2005).

Family Coccinellidae was first recorded in Systema Naturae (Linnaeus 1758) under genus Coccinella with 36 species in which many are still valid species. There are only limited study on the predatory beetles and their prey species, particularly ladybird beetles and aphids in Nepal. However, the sporadic taxonomic studies were published (Kapur 1955, Miyatake 1967, 1985, Canepari and Milanese 1997, 2012, Canepari 2003, Bista and Omkar 2011, Thapa 2015, K.C. et al. 2018, 2019).

1.2 Objectives of the study

The general objective of this study was to explore species diversity of ladybird beetles, their associated aphid prey, relation with temperature in Tribhuvan University premises, Kirtipur and compare feeding efficiency among two predator coccinellids.

The specific objectives were;

i. To explore species diversity of ladybird beetles within Tribhuvan University premises, Kirtipur.
ii. To find association of ladybird beetles with aphid prey species in the study area.
iii. To find relation of coccinellids with temperature.
iv. To examine feeding efficiency of ladybird beetle *Coccinella septempunctata* and *Propylea luteopustulata* against aphids.

1.3 Rationale of the study

Ladybird beetles are economically very important insect predators which are used as model organisms for biological control agents in the world (Kindlmann and Dixon 1999b). There are only a few studies published on ladybird beetles of Nepal (Kapur 1955, Miyatake 1967, 1985, Canepari and Milanese 1997, 2012, Thapa 2015). But there was a lack of data for the complex landscape of Tribhuvan University. The predator prey association can offer baseline data for more studies that can assist in biological control in agricultural systems (Inayat et al. 2011). They are used to control aphids which are serious pests of many crops and fruits. But the association of ladybird beetles and aphids are not yet documented in Nepal which is very important to know which beetle species consume which species of aphids. This kind of information will be important to identify the efficient biological control agents. So, this study found the species diversity of ladybird beetles and associated aphid fauna of Nepal with reference to the university owned land of Tribhuvan University, Kirtipur which fulfilled this information gap.

1.4 Limitations of the study

The general limitations of this study were as follows;

- Readings about humidity and precipitation were taken by using a hygrometer and weather application. These data were found highly variables which were not matched. Considering high errors, humidity and precipitation data were excluded in present analysis.

2. LITERATURE REVIEW

2.1 Species Diversity of Coccinellids

There are about 6000 species and 360 genera of ladybird beetles in the world (Vandenberg 2000, Slipinski 2007). In Nepal, there are 235 species of ladybird beetles belonging to 57 genera and six subfamilies including 26 endemic species (Thapa 2015). There are several studies carried in Nepal and surrounding countries in different aspects of ladybird beetles and aphids such as taxonomy, distribution, diversity, biology, ecology and pest control.

In Nepal, Hope (1831) initiated study of ladybird beetles giving 19 new species. Study of coccinellids of Nepal were carried out by researchers from different parts of world (Mulsant 1850, Crotch 1874, Dohrn 1882, Kapur 1955, Miyatake 1985, Bielawski 1971, 1972, Canepari and Milanese 1997, Canepari 2003). A recent survey conducted on the predatory ladybirds in Nepal included Kanchanpur district (Bista and Omkar 2011), Lamjung and Kaski districts (K.C. et al. 2018), Lalitpur district (K.C. et al. 2019). Recently, taxonomic works were carried out in two hilly districts viz., Sundarbazar, Lamjung and Pokhara, Kaski from altitudes ranging from 700-830 masl with first record of *Jauravia assamensis* which was recorded only from India (Assam) before (K.C. et al. 2018). The exclusive research from Nepal Agricultural Research Council, Khumaltar, Lalitpur identified and gave description of 14 species from 9 genera (*Calvia, Cheilomenes, Coccinella, Coelophora, Harmonia*, *Hippodamia*, *Illeis*, *Oenopia* and *Propylea*) along with its food and host plants (K.C. et al. 2019).

The survey of coccinellids in the Chitral District, Pakistan in 2001 found twelve different species from altitudes of 1219.40 – 2651.63 m in a wide variety of terrestrial habitats (Khan et al. 2007). The total of 15 species belonging to subfamilies Coccinellinae and Chilocorinae from fruit, cruciferous crop, forest and flower ecosystems were identified from Kashmir, India from April- September (Khan et al. 2009). A survey in fruit orchards and oak forest of Uttarakhand, India showed 23 coccinellid species with 8 species recorded for the first time from the study area (Joshi et al. 2012). Chakrabarti et al. (2012) found that species diversity was greater in the agro ecosystem than the forest ecosystem. Raikar (2013) investigated coccinellid of fauna at Main Agricultural Research Station Dharwad in three different ecosystems viz., agricultural, horticultural and organically grown crops found 18 different species. *C. sexmaculata* was most dominant predatory coccinellid species in all the three crop ecosystems even species diversity of Horticulture ecosystem was higher than agriculture and organing farming block. The study in the Hamedan district, Iran recorded twelve species belonging to 3 subfamilies with high abundance as well as wide distribution of *Hippodamia variegata* with highest Simpson's diversity index of 0.55 during September (Akhavan et al. 2013). The investigation in seasonal changes of adult coccinellids in central Europe species varied throughout vegetative season with high abundance of each species in a period of time (Honek et al. 2015). The study of different beetles over two years in different elevational zones of Binsar Wildlife Sanctuary in district Almora, Uttarakhand, India showed *C. septempunctata* (16.62%) most abundant species (Arya et al. 2016).

The extensive faunal survey in the district Sargodha at 544-633 ft found nine species belonging to 9 genera and 4 subfamilies out of total 75 species found in Pakistan occurring along 10 trophic associations among which *C. septempunctata* was widely distributed (Ahmed et al. 2017). Albaaj (2017) explored biodiversity of coccinellids in soyabean agroecosystems at Wood county, Ohio in 2016 with eight species among which *Hippodamia convergens* (72%) was most dominant and the Shannon diversity index value and Shannon evenness value were 1.516 and 0.729 respectively. According to Hayat et al. 2017, 2 species in 2 genera of subfamily Coccidullinae and 30 species in 18 genera of Coccinellinae were found in Azad Jammu and Kashmir, Pakistan. Lorestan province (Western Iran) includes total 57 species belonging to 25 genera of 10 tribes collected during 2013-2016 as well as from review on Iranian ladybirds (Biranvand et al. 2018). The study in hazelnut orchards of Turkey showed the highest coccinellid population in April and May and a decreasing trend till the end of October (Tuncer et al. 2018).

Abundance and diversity of ladybeetles were of unique species responding in opposite directions to urbanization in gardens of two US regions having higher diversity in California with dry summer than Michigan with relatively wet summer (Egerer et al. 2018). Sampling done from rainfed and irrigated fields of Punjab, Pakistan resulted in greater diversity and abundance in irrigated fields than in rainfed fields (Hussain et al. 2017).

2.2 Ladybird beetles-aphids association

The prey predator catalogue of 36 true aphidophagous coccinellids was reviewed along with incidental predators, unidentified or doubtful records (Agarwala and Ghosh 1988). Similarly, predator-prey catalogue of 251 known predaceous coccinellids of India was presented along with all the prey range in addition to aphids (Omkar and Pervez 2004). Rafi (2008) listed coccinellids of aphids in his dissertation along with other predators and parasitoids. *C. septempunctata*, *C. undecimpunctata, C. sexmaculata H. variegata* and *Calosoma maderae* were significantly associated with *Schizaphis graminum* in Brassics, wheat and fodder in central Punjab, Pakistan (Inayat et al. 2011). Aphidophagous predator coccinellids of eastern Himalayas and Northeast India along with their prey species were recorded (Chakrabarti et al. 2012). However, the study carried out on the coccinellid population in different crops had significant and positive correlation with aphids (Raikar 2013). Twelve species of coccinellids associated as natural enemies of aphids and coccids were recorded from Sikkim, India (Joshi and Sangma 2015). The investigation of aphid prey of *C. sexmaculata* in an urban park in central Japan showed association with five aphid species (Kawakami et al. 2016).

In Foraging Behaviour of Predaceous Ladybird Beetles, allelochemical from aphid plant complexes guide ladybird to aphid site with predator prey association was reviewed (Pervez and Yadav 2018). The study was undertaken to assess prey on the rice crop and surrounding flora in Hyderabad, India recorded and analyzed coccinellids feeding their items (Shanker et al. 2018).

Six coccinellid natural enemies of *Aphis punicae* infesting pomegranate in Kashmir were recorded (Mohi-ud-din et al. 2019). Rasool et al. (2019) surveyed Budgam district of Kashmir valley and found eight predators *Chilochorus infernalis, Adalia tetraspilota, H. variegata*, *C. septempunctata*, *Harmonia spp., Oenopia conglobata*, *Syrphus* spp. and *Chrysoperla zastrowi* associated with wooly apple aphid (*Erisoma lanigerum*) in apple orchards. The survey of natural enemies associated with *Aphis punicae* in Kashmir, India showed that six coccinellid predator’s viz. *C. septempunctata, Harmonia eucharis, C. sexmaculata*, *Adalia tetraspilota, H. variegata* and *Calvia punctata* were associated with pomegranate aphids (Hassan et al. 2019). Gurung et al. (2019) also surveyed predaceous coccinellids under different crop ecosystems in hilly and terai region of West Bengal along with associated prey.

Ghani and Maalik (2020) during study of insect fauna associated with *Triticum aestivum* in Sialkot, Pakistan found *C. septempunctata* showed significant association with most of its preys like *D. noxia* followed by *S. graminum* and *R. padi*, *C. sexmaculata* was also significantly associated with *S. graminum*. The study carried out in Adana Province of Turkey showed 28 species belonging to 20 genera associated with 42 pest species among which twenty species were recorded to be primarily feeding on aphids (Elekcioglu 2020).

Based on the literature searched from 1984 to 2020 (Note- Omkar and Pervez 2004, Conway and Kring 2010, Chakrabarti et al. 2012, Prabhakar and Roy 2010, Elekcioglu 2020, Rafi 2008, Ahmad et al. 2017, Bhagat et al. 1988, Ghani and Maalik 2020, Inayat et al. 2011, Mohi ud din et al. 2019, Zhu and Park 2005, Pervez and Yadav 2018, Raikar 2013, Shanker et al. 2018, Kawakami et al. 2016, Omkar and Mishra 2005c, Agarwala and Ghosh 1988, Gurung et al. 2019, Joshi and Sangma 2015, Poorani and Kumar 2018) on the selected ladybird beetle species, pest aphids on different crops, most of them are generalists feeding on wide range of aphid preys (Table 1).

Table 1. Coccinellids with their associated aphids

Coccinellid	**Aphids**
C. septempunctata	1,2,5,6,7,8,9,12,14,15,16,17,21,23,24,25,26,28,32, 35,39,40.44,45,55
C. sexmaculata	2,5,8,10,12,13,16,17,21,26,28,31,32,33,35,38,39,40,42,43,44,46,47
C. transversalis	2,5,6,7,8,10,16,17,26,28,35,38,39,40,41,42,44,46, 48,56
H. variegata	1,2,5,8,12,14,15,16, 17,19,20,25,29,34,35,36,40,42,46
P. dissecta	2,5,6,8,10,17,22,26,35,38,42,46
O. kirbyi	1,2,7,17,18,22,28,32,35,38,44,49,50,56
O. quadripunctata	1,2,44

O. sauzeti	1,2,3,4,7,11,16,17,18,22,27,28,30,31,32,33,35,37, 44,51,52,53,54,56
O. mimica	2,55
P. luteopustulata	2,5,8,16,18,22,26,28,31,32,35,38
C. bissellata	2,8,38,45,56
H. sedecimnotata	8,35

Note to Table 1- 1= *A. fabae*, 2= *A. gossypii*, 3= *A. kurosawai*, 4= *A. longisetosa*, 5= *Acrythosiphon pisum*, 6= *A. affinis*, 7= *A. citricola*, 8= *A. craccivora*, 9= *A. glycines*, 10= *A. nerii*, 11= *A. pomi*, 12= *A. punicae*, 13= *A. spiraecola*, 14= *Aulacorthum solani*, 15= *Brachycaudus (Acaudus) cardui*, 16= *B. helichrysi*, 17= *Brevicornye brassicae*, 18= *Capitophorus formosartemisiae*, 19= *Cavariella theobaldi*, 20= *Cryptomyzus ribis*, 21= *Diuraphis noxia*, 22= *Eriosoma lanigerum*, 23= *Eucarazzia elegans*, 24= *Hyadaphis coriandri*, 25=*Kaltenbachiella pallida*, 26= *L. erysimi*, 27=*Liosomaphis atra*, 28= *M. (Sitobion) rosaeiformis*, 29= *Macrosiphoniella abrotani*, 30= *M. pseudoartemisiae*, 31= *M. sanborni*, 32= *Macrosiphum rosae*, 33= *Melanaphis donacis*, 34= *Microlophium carnosum*, 35= *Myzus persicae*, 36= *Ovatus mentharius*, 37= *Phorodon cannabis*, 38= *Rhopalosiphum maidis*, 39= *R. padi*, 40= *Schizaphis graminum*, 41= *Sitobion sp*, 42= *Therioaphis maculate*, 43= *Tinocallis kahawaluokalani*, 44= *Toxoptera aurantii*, 45= *T. odinae*, 46= *Uroleucon compositae*, 47= *Uroleucon nigrotuberculatum*, 48= *Uroleucon sp.*, 49= *A. paraverbasci*, 50= *Coloradoa rufomaculate*, 51= *C. hippophaes*, 52= *Cavariella aegopodii*, 53= *Clethrobius dryobius*, 54= *Coloradoa artemisiae*, 55= *Taoia indica*, 56= *M. yomogifoliae.*

2.3 Relation of coccinellids with weather parameters

The temperature and relative humidity had a negative significant effect, rainfall had non-significant negative relation with coccinellidae at Agricultural Research substation at Rajasthan, India (Dhaka and Pareek 2007). The predator population (*C. septempunctata*) had positive significant correlation with mean temperature, sunshine and vapour pressure and non-significant relation with rainfall and relative humidity under wheat agroecosystem (Soni et al. 2013). The study carried out on the coccinellid population in five different crops had significant and positive correlation with aphids but negative correlation with minimum temperature and rainfall. The coccinellid population had positive correlation with relative humidity in sorghum, maize and safflower whereas it was negative in cowpea and Lucerne (Raikar 2013). Ghosh et al. (2013) from India in rice crop found significant positive influence of temperature and relative humidity and rainfall imparted insignificant positive influence on *C. septempunctata* population.

Shukla (2014) reported significant negative correlation of coccinellids with maximum temperature and relative humidity in the afternoon, non-significant negative correlation with minimum temperature and relative humidity in the morning whereas positive non-significant relation with rainfall on okra. The correlation analysis done on coccinellid population under mustard agroecosystem revealed coccinellid population had a positive correlation with temperature, whereas a significant negative correlation with relative humidity at 0.05 per cent level (Bajia and Singh 2014).

Ghortale et al. (2016) who observed seasonal dynamics of wheat aphid and coccinellid reported that maximum temperature and rainfall had non-significant negative correlation and positive non-significant relation with coccinellid. Boda and Ilyas (2017) showed maximum temperature had significant positive effect, minimum temperature had non-significant effect, precipitation had negative whereas relative humidity had non-significant negative effect on ladybird on cotton ecosystem at the Experimental Farm, Department of Agricultural Entomology, VNMKV, Parbhani.

The maximum and minimum temperature had significant positive relation with coccinellid (*C. septempunctata*) population in cauliflower (Jakhar and Singh 2018).Similarly, Gurung et al. (2018) showed significant negative association of coccinellids with relative humidity and insignificant association with temperature and rainfall in brinjal and wheat plants. According to Mohapatra et al. (2018), *C. septempunctata* and *C. sexmaculata* on *Vigma mungo* had non-significant negative correlation with minimum temperature and rainfall, significant negative correlation with relative humidity and non-significant positive correlation with maximum temperature. Observation by Swami et al. (2018) revealed non-significant effects of maximum and minimum temperature, average relative humidity and rainfall on coccinellid in coriander. Kedar et al. (2018) from correlation studies observed highly significant negative correlation with maximum temperature, minimum temperature and wind speed whereas evening relative humidity, Sunshine hours and rainfall had non-significant positive relation with coccinellidae in the cotton ecosystem.

Harshita et al. (2019) observed non-significant positive relation of coccinellid abundance with temperature and rainfall while significant negative relation with relative humidity in tomato ecosystem. Pradhan et al. (2019) revealed that maximum temperature had non-significant positive relation and rainfall had non-significant negative relation with *C. transversalis* in mustard. Similarly, Ladybird beetles showed positive relation with temperature and negative relation with temperature in cotton (Ramzan et al. 2019).

2.4 Feeding efficiency of ladybirds

The study showed *C. septempunctata* had high predation rate on *L. erysimi* followed by *Hyadaphis coriandri, Rhopalosiphum nymphae, A. craccivora* and *Macrosiphum rosae,* also showed that predation was significantly different with respect to all aphid species under 25±°C, 70±5% RH, 12hL: 12hD (Ali and Rizvi, 2007). The most preferred temperature for survival and fecundity of *C. septempunctata* reared on *L. erysimi* are 28±1°C and 24±1°C respectively which help in mass rearing and biological control program (Ali and Rizvi, 2008). Godeau et al. (2008) performed experiments with myrmecophilous *Coccinella magnifica* and non myrmecophilous *C. septempunctata* feeding biology on *Symydobius oblongus* resulting that *C. magnifica* had higher predation efficiency than *C. septempunctata* in the both presence/ absence of ants.

Prabhakar and Roy (2010) showed that male and female *C. septempunctata*, *C. transversalis, C. sexmaculata, M. discolor* and *Pullus pyrocheilus* have high consumption rate on *A. craccivora* (65.6±3.01, 52±4.2), on *L. erysimi* (57±4.4, 41.25±1.7), on *A. gossypii* (57±2.26, 39.5±0.55), on *Myzus persicae* (43.4±0.51, 30.66±0.62) and on *L. erysimi* (34.75±1.4, 25.2±0.65) respectively and on nymphs of aphids in comparison to their adults was due to the fulfillment of dietary requirements of these predators from these prey species. According to the Rai et al. (2010) functional response of coccinellids on *L. erysimi, C. septempunctata* was found to have significant preying capacity than *C. transversalis* in different densities of aphid.

The study on the predation rate of variegated lady beetle, *H. variegata* fed on the black bean aphid, *Aphis fabae* showed that females had higher predation than male (Farhadi et al. 2011). The potential of four co-occuring ladybirds *C. septempunctata, C. transversalis,* *C. sexmaculata and P. dissecta* as predators of the pea aphid, *A. pisum* showed larger ladybirds were more voracious while smaller ones had higher consumption indices and were more efficient converters of food, but no species performed well in terms of all measures of predatory potential assessed (Mishra et al. 2011). Larger ladybirds' C. *septempunctata* and *C. transversalis* had higher consumption rate on *A. pisum* than smaller *C. sexmaculata* and *P. dissecta* (Mishra et al. 2012). The study on functional responses resulted in significantly greater voracity of *C. transversalis* than *P. dissecta* even at different prey densities (Omkar and Pervez 2011).

B. suturalis reared group or solitary had no influence on overall development as well as survival but prey species had significant influence. *B. suturalis* had faster development and higher survivability on *A. pisum* than on *A. gossypii* (Bista et al. 2012). The study of functional response of all stages of *C. septempunctata* against two species of aphids *L. erysimi* and *Brevicoryne brassicae* infesting mustard and cabbage crops showed that *L. erysimi* is preferred and adult female was most efficient among all stages (Gupta et al. 2012).

The laboratory experiments conducted on fourth instar larvae, adult male, and adult female ladybird beetles *A. cardoni* Weise, to different densities of aphids, *A. gossypii* Glover (Hemiptera: Aphididae), on the bottle gourd, *Lagenaria vulgaris* showed that fourth instar larvae of *A. cardoni* may be considered the most efficient predatory stage in aphid management strategies. Prey consumption was highest and handling time lowest in fourth instar larvae, followed by adult females and males (Omkar and Kumar 2013). The predatory performance of four predators showed that *C. septempunctata* had highest voracity among *C. transversalis*, *C. sexmaculata*, *P. dissecta* on aphids viz., *L. erysimi* and *A. craccivora* but performed better on *L. erysimi* where others performed better on *A. craccivora* (Kumar et al. 2013).

The modeling done by comparing the consumptive and non-consumptive effect of *C. septempunctata* and *Coccinella novemnotata* on *A. pisum* foraging faba beans indicated that *C. septempunctata* had greater predatory efficiency (Hoki 2014). The consumption capacity of *Harmonia axyridis* using *Cinara atlantica* in different stages showed that the fourth larva feeding 46 ± 6.8 aphids per day is efficient among all stages (Santos et al. 2014). The study done between two congeneric ladybirds *C. septempunctata* and *C.*

transversalis reared in *A. pisum* showed higher consumption rate and growth rate in *C. septempunctata* than in *C. transversalis* (Kumar et al. 2014). *C. undecimpunctata* and *Hippodamia tredecimpunctata* in Saudi Arabia provided with two prey species *A. gossypii* and *Aphis punicae* suggested *C. undecimpunctata* as efficient predators of both aphid species (Al-Deghairi et al. 2014).

The investigation in effect of prey resource exploitation of *A. pisum* resulted *Menochilus sexmaculatus* as predator with higher ability to suppress aphid than *P. dissecta* in all (scarce/optimal/abundant) conditions (Chaudhary et al. 2015). The predation efficiency of *C. septempunctata* on *M. rosae* in Kastamonu, Turkey showed that the fourth larva is most voracious and efficient among all stages (Unal et al. 2017). According to experiment done by Sarmad et al. (2015), *C. septempunctata* is efficient as compared to *Propylea quatuordecimpunctata* against *M. rosae* under laboratory conditions (25±°2C, 65±5% R.H.).

Jesu Rajan et al. (2018) reported that the study on consumption rate of coccinellid predator, *C. transversalis*, on cabbage aphid, *B. brassicae* in the biocontrol laboratory showed 4th instar grubs consumed significantly more aphids when compared to 1st, 2nd and 3rd instars. Predation rate of grub (total grub period) and adult was 75 - 84 and 48 - 50 aphids/ day. Maharjan et al. (2019) conducted laboratory studies which concluded *C. septempunctata* were better predators than *Adonia variegata*. The experiment showed that *C. septempunctata* had highest consumption on *L. erysimi* followed by *M. persicae*, *B. brassicae* and *A. craccivora*.

3. MATERIALS AND METHODS

3.1 Study Area

Tribhuvan University area at Kirtipur, Kathmandu, Nepal spreads over an area of 154.77 hectares. The study was carried out in different habitats of T.U. with geographic coordinates 27°40'–27°41' N, 85°16'–85°17' E, and elevation at 1280-1350 masl. Common vegetation found were *Parthenium hysterophorus, Artemisia vulgaris, Rosa chinensis, Tagetes erecta, Sigesbeckia orientalis, Oryza sativa, Ageratina adenophora, Zinnia elegans, Urena lobata, Zea mays, Coriandrum sativum, Xanthium strumarium, Circium arvense, Psidium guajava, Paspalum dilatatum, Juniperus* sp., *Pinus roxburghii* during study period. The different sites selected for the collection and study purpose are:-

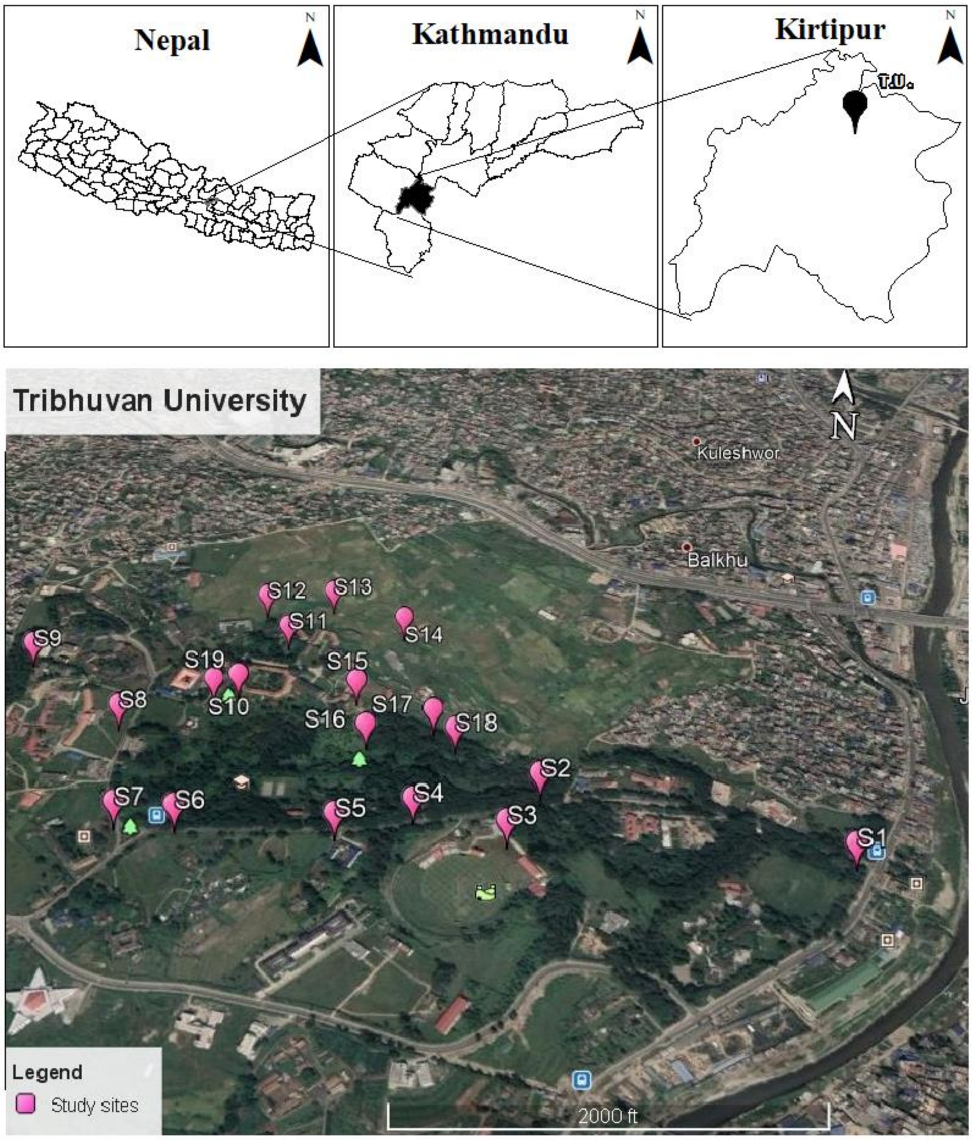

Figure 1: Sampling sites of Coccinellids in Tribhuvan University area, Kirtipur

3.2 Materials

Ladybird beetles sampling and preservation - Cotton, vials, camera, hygrometer, labels, entomological pins, entomological box and Naphthalene balls

Lab work- Stereoscopic microscope, Graph paper, Spirit lamp, test tube, test tube holder, matchstick, slides, coverslip

Feeding experiment- Petri dishes, blotting papers, Camel hair brush

Chemicals used- Ethyl acetate, Potassium hydroxide, alcohol

3.3 Methods

3.3.1 Sampling methods

Altogether nineteen sites were selected randomly with varied characteristic features viz. agricultural land (4 spots), grassland (7 spots), garden (2 spots) and forest area (6 spots). Beetles and aphids were monitored repeatedly from 9:30 am to 5pm at an interval of 15 days from 14th May to 9th November in 2019 (summer, rainy and autumn) in 19 selected sites in Tribhuvan University premises (Figure 1). Summer (Mid May – Mid July), Rainy (Mid July – Mid Sep) and Autumn (Mid Sep to Mid Nov) were classified for sampling periods on the basis of Nepali calendar. Visual observation for 20 minutes was done in each site within a 100 m^2 area. The number of ladybird beetles were counted on the field and selected specimens were collected by hand collection method. Each sampling site was geo-located using Global Positioning System (GPS) coordinates application.

To study association of aphid and beetles, both species were picked up by hands from the same infected plant part (Jonathan 1995). All collected samples were brought to the lab for identification. Temperature were also recorded in each site immediately after observation.

3.3.2 Preservation and pinning

The collected insects were transferred into air tight labelled jars containing ethyl acetate soaked cotton. Specimens were transferred into separate containers and brought to the laboratory and pinned with proper labeling including scientific name, GPS location, collection date, collector’s name and catalogue number. Small sized specimens were mounted on a small rectangular card. Large specimens were pinned directly on the right elytra just behind the pronotum. The specimens were then kept in wooden insect boxes for permanent storage with naphthalene balls **(**Ashfaque 2012). Permanent slides of aphids were prepared for identification and permanent storage (Sirisena et al. 2013).

3.3.3 Identification and Photography

The collected specimens were identified with the help of description and figures using Mulsant 1850, Kapur 1946, Kapur 1955, Bielawski 1972, Kapur 1973, Miyatake 1985, Canepari and Milanese 1997, Poorani 2002, Hayat et al. 2017, Janakiraman and Thangjam 2019 for ladybird beetles, and using identification keys: Stoetzel and Miller 2001, Blackman and Eastop 2020, Das and Raychaudhuri 1983 for aphids, photographed and labeled. Photographs were taken using mobile camera of Gionee model and Nikon D50. Final photoplate of species was prepared by using Adobe Photoshop CC 14.2.1. All samples were deposited in the Central Department Zoology Museum of Tribhuvan University (CDZMTU) with catalogue numbers.

3.3.4 Feeding efficiency experiment

Individuals of *C. septempunctata* and *P. luteopustulata* were collected from T.U. a few days before the experiment. Mustard aphids were collected regularly from mustard plant twigs. To study feeding efficiency, selected species of predator i.e. *C. septempunctata* and *P. luteopustulata* and aphids of Mustard were taken in the room temperature at Patan, Lalitpur from 29th December (2019) to 15th March (2020). Beetles were starved for 24 hours to standardize their physiological status. For each species, five replicates of a petri-dish of 8.5 cm diameter × 1 cm size were used for feeding experiments. Blotting paper was placed at the bottom of each petri-dish and replaced whenever necessary. Daily, 100 aphid prey along with the fresh twigs and flowers of the respective host plants were placed in each petridish. Unconsumed aphids were counted daily in each petri-dish within 24 hrs to evaluate the consumption rate (Prabhakar and Roy 2010).

3.3.5 Data analysis

Shannon diversity (1948), Pielou's evenness index (1966) for diversity and mean ± S.E. feeding efficiency was calculated in Microsoft excel 2013. Relation of coccinellid abundance with weather parameter (temperature) was analysed using Generalized linear model with Poisson distribution in R program (Rstudio team 2020).

4. RESULTS

4.1 Diversity of ladybird beetles

A total of 17 species (Fig. 3) under 11 genera of Coccinellidae were identified from 567 individuals belonging subfamily Coccinellinae and four tribes (Coccinellini, Epilachni, Sticholotini and Noviini) from the study area. The overall calculated Shannon Diversity index (H) was 1.25 (Appendix I). Among total 567 beetles individuals collected, *C. septempunctata* (71.78%) was most abundant species. (Fig. 2). All the species with its abundance is shown (Fig. 3).

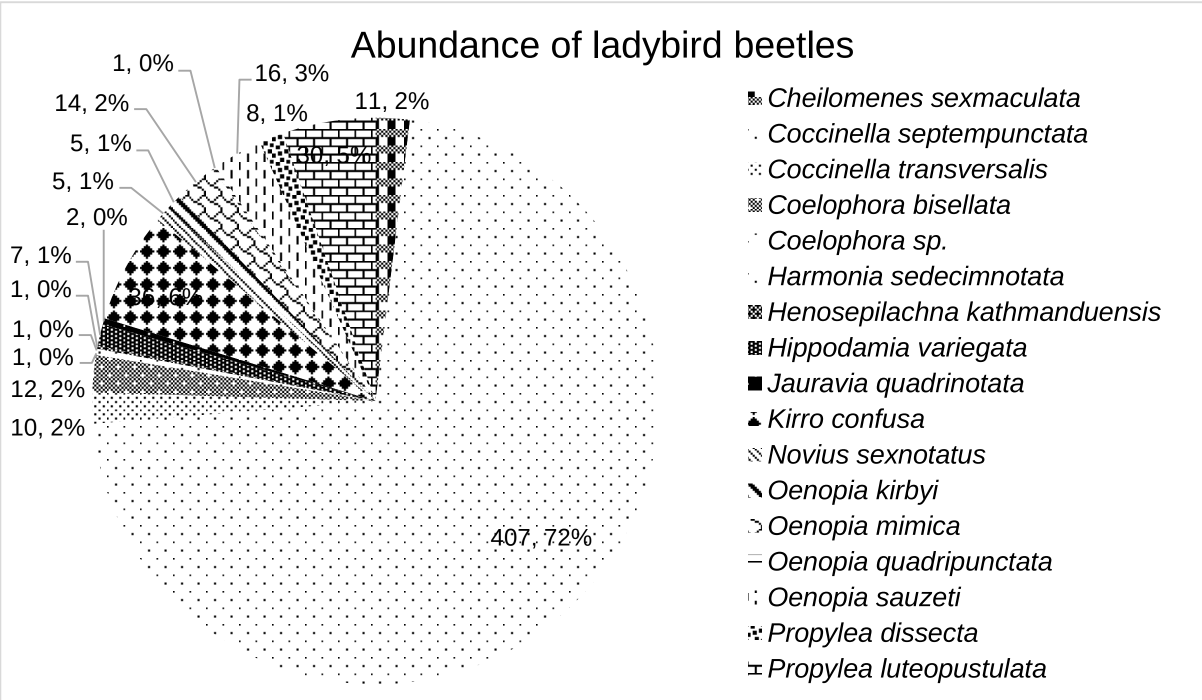

Figure 2: Abundance of ladybird beetles

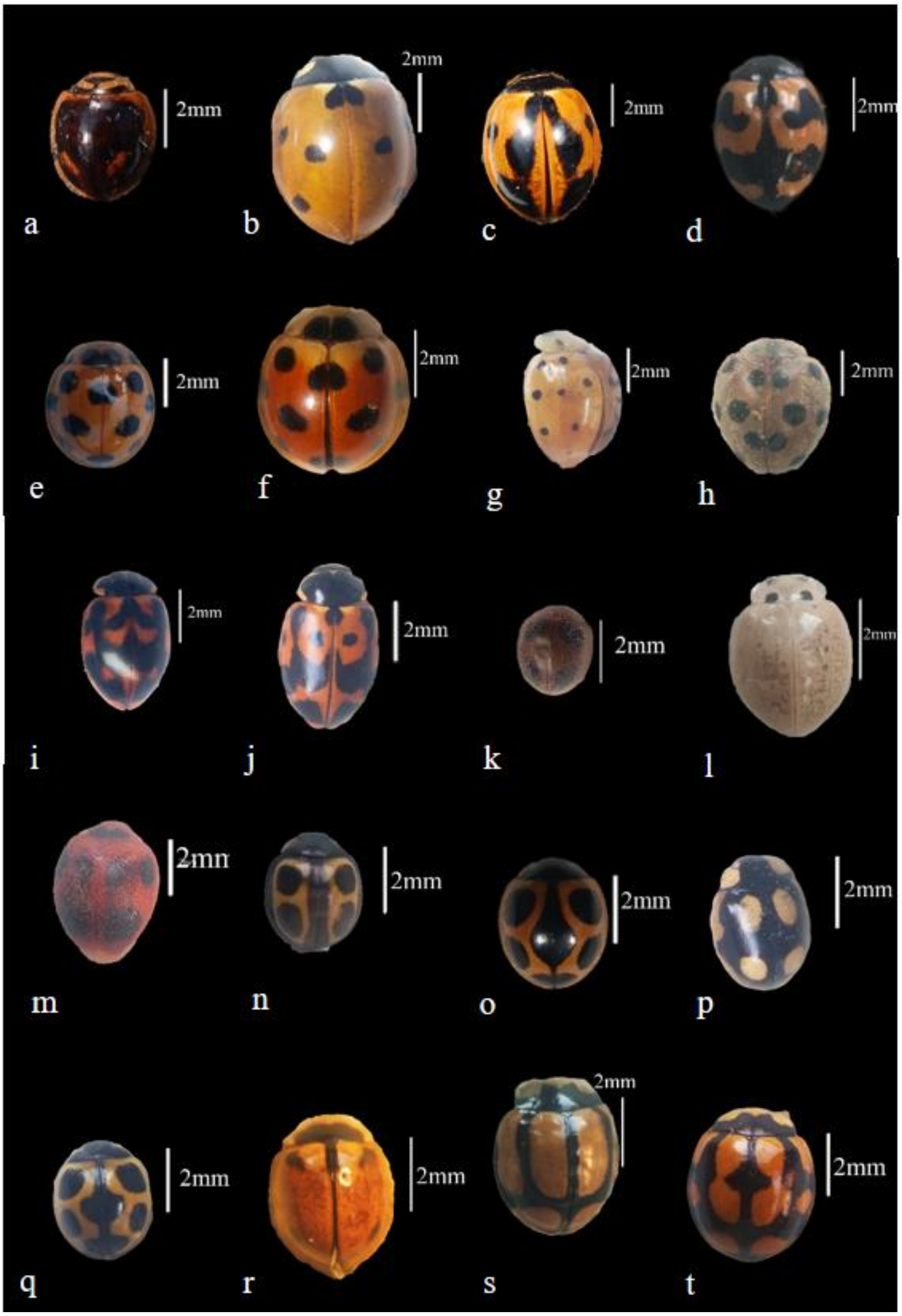

Figure 3: Different species of ladybird beetles with catalogue number a. *C. sexmaculata*: CDZMTU-COL01, b-c. *C. septempunctata*: CDZMTU-COL02, d. *C. transversalis*: CDZMTU-COL03, e. *C. bissellata*: CDZMTU-COL04, f. *Coelophora* sp.: CDZMTU-COL05, g. *H. sedecimnotata*: CDZMTU-COL06, h. *H. kathmanduensis*: CDZMTU-COL07, i-j. *H. variegata*: CDZMTU-COL08, k. *J. quadrinotata*: CDZMTU-COL09, l. *K.*

confusa: CDZMTU-COL10, m. *N. sexnotatus*: CDZMTU-COL11, n. *O. kirbyi*: CDZMTU-COL12, o. *O. mimica*: CDZMTU-COL13, p. *O. quadripunctata* : CDZMTU-COL14, q. *O. sauzeti*: CDZMTU-COL15, r. *P. dissecta*: CDZMTU-COL16, s. *P. luteopustulata*: CDZMTU-COL19, t. *P. luteopustulata*: CDZMTU-COL17

4.1.1 Seasonal Diversity of ladybird beetle within T.U. premises

The diversity of ladybird beetles was highest in autumn season with H=1.51 as compared to rainy season with H= 0.96 and summer season with H= 0.85 (Table 2). *H. sedecimnotata* and *O. quadripunctata* were found in summer only whereas *H. kathmanduensis*, *K. confusa* and *P. dissecta* were found only in autumn season (Fig. 4).

Table 2: Diversity and evenness in different seasons

	Summer	Rainy	Autumn
Richness	12	11	12
Abundance	270	110	187
Shannon Diversity Index (H)	0.85	0.96	1.51
Evenness (J)	0.34	0.40	0.61

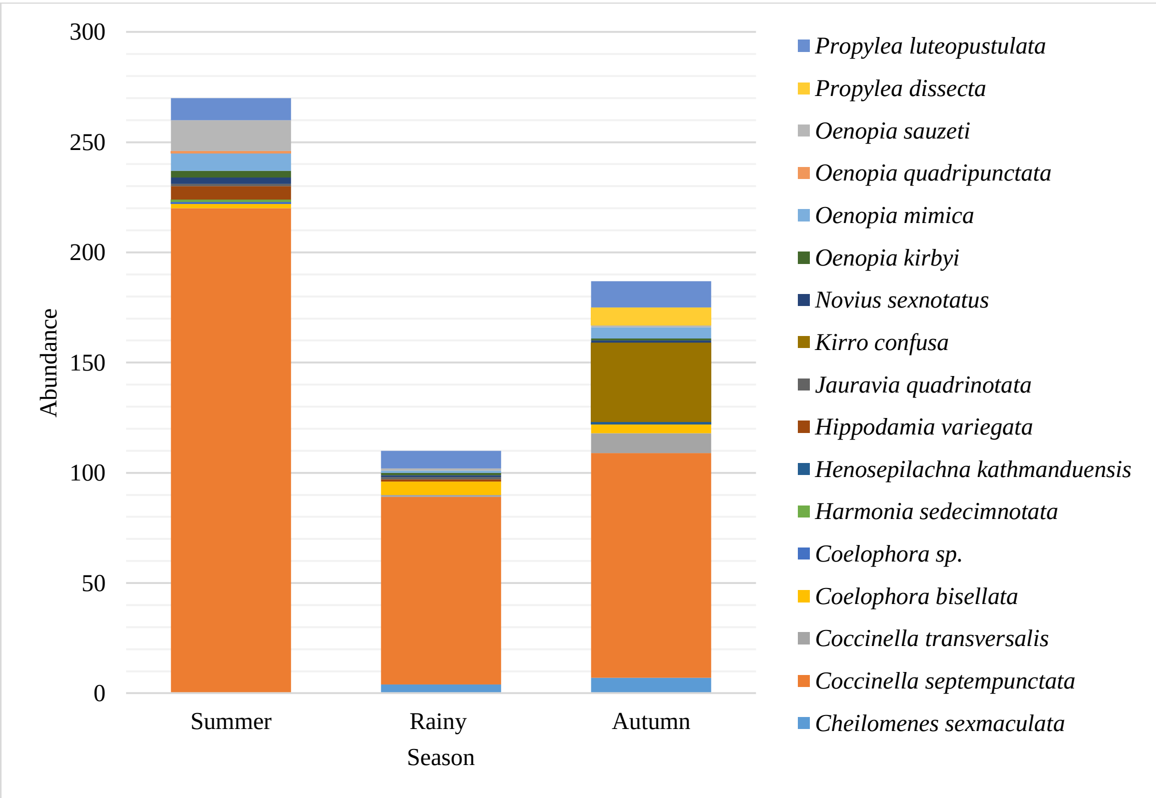

Figure 4: Ladybird beetle abundance in summer, autumn and rainy

4.1.2 Habitat wise Ladybird Beetle Diversity in T.U., premises

Shannon Diversity Index of Ladybird beetle was highest in agricultural land (H=1.44, J=0.56) followed by garden (H=1.36, J=0.69), planted forest (H=1.25, J=0.70) and least in grassland (H=0.96, J=0.38) (Table 3). Among them, *C. transversalis, H. sedecimnotata* were found only in grassland, *H. kathmanduensis, J. quadrinotata* found only in garden, *N. sexnotatus* and *O. quadripunctata* were found only in agricultural land whereas *C. septempunctata* was found in all the sites (Fig. 5).

Table 3: Diversity and evenness in different habitats

	Agricultural land	Garden	Planted forest	Grassland
Richness	13	7	6	12
Abundance	114	39	74	340
Shannon Diversity Index (H)	1.44	1.36	1.25	0.96
Evenness (J)	0.56	0.69	0.70	0.38

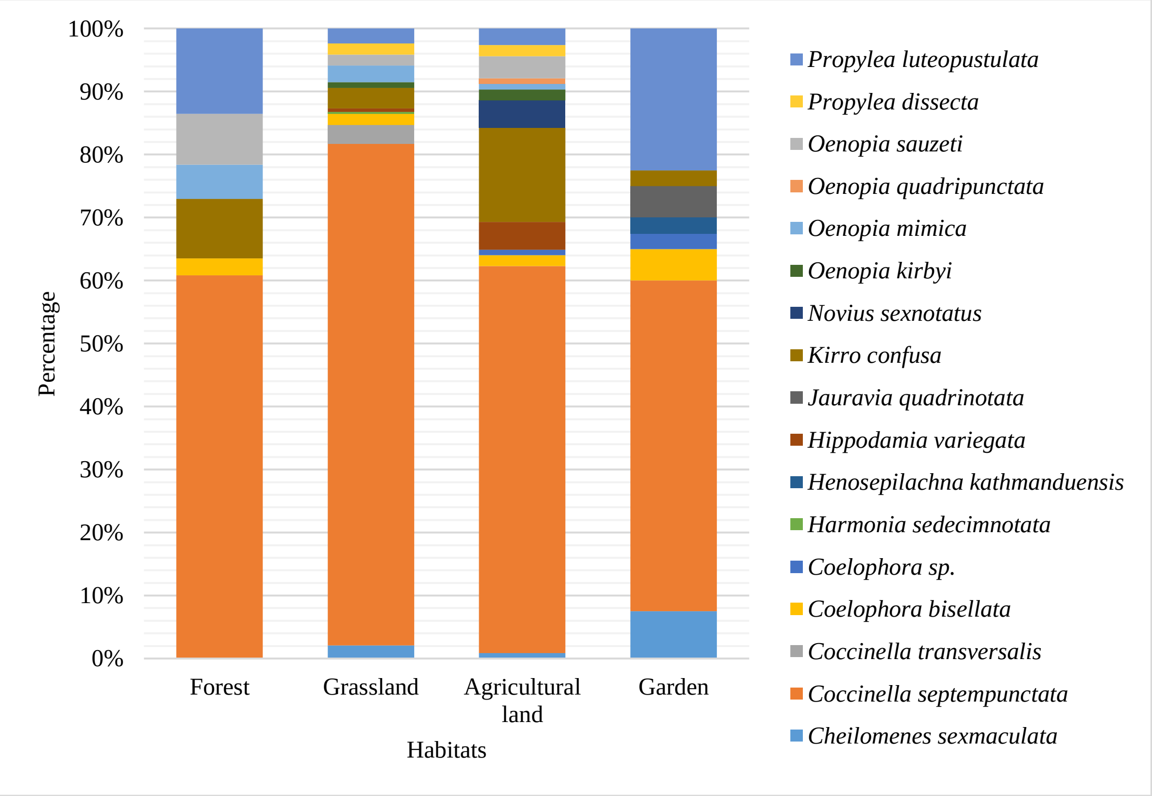

Figure 5: Ladybird beetle abundance in different habitats

4.2 Association of ladybird beetles and aphid species

The listed coccinellids were associated with aphid species. *C. septempunctata* has a wide food range and is associated with 5 aphid species found in three host plants. Some are species specific such as *Cheilomenes sexmaculata* were found with *M. rosae*; *P. luteopustulata* with *A. gossypii; O. sauzeti, O. mimica* were found with *M. yomogifoliae* in host plant *A. vulgaris* in this study (Table 4).

Table 4: Coccinellid species and their associated aphid prey

Coccinellid	**Aphid**	**Host plant**
C. septempunctata	*Macrosiphoniella pseudoartemisiae, M. yomogifoliae, Rhopalosiphum maidis, Aphis gossypii, Macrosiphum rosae*	*Artemisia vulgaris, Zea mays, Rosa chinensis*
P. luteopustulata	*A. gossypii*	*A. vulgaris*
O. sauzeti	*M. yomogifoliae*	*A. vulgaris*
O. mimica	*M. yomogifoliae*	*A. vulgaris*
C. sexmaculata	*M. rosae*	*R. chinensis*

4.3 Effect of weather parameters on coccinellids abundance

Log mean count of ladybird beetles was the highest in the summer. Least mean count was found in the rainy season which is significantly lower than summer season ($p<0.05$). Only marginal significance between summer and autumn season was seen ($P=0.05$). Addition of temperature improved the fit of the model after accounting for the season. The model predicted the rise of log mean count of the beetle was 0.03 times for each degree rise in temperature (Table 5).

Table 5. Generalized linear model with Poisson distribution selected according to the Akaike information criterion adjusted for small sample size (AICc). Model parameters include season and temperature, ΔAICc is the difference between the AICc value of the best-supported model and successive models, and wi is the Akaike model weight.

Model parameters	**K**	**loglink**	**AICc**	**ΔAICc**	**Wi**
Season + Temperature	4	-697.40	1402.80	0.00	0.76
Season	3	-699.56	1405.13	2.33	1.00
Null	1	-734.34	1470.68	67.88	1.00

Among these variables, the probability of finding beetles in the rainy season is significantly less than autumn, but in the summer season the probability of finding them is significantly higher. Similarly, temperature showed a positive effect on abundance of ladybird beetles. With increase in the temperature, the abundance of beetles increased (Table 6).

Table 6. Model-averaged parameter describing effect of weather parameters.

Parameters	**Estimate**	**SE**	**Pr(>|z|)**
(Intercept)	0.40507	0.42734	0.3432
Autumn	-0.22793	0.11773	0.0529.
Rainy	-0.89581	0.11314	2.42e-15***
Temperature	0.03226	0.01572	0.0402*

Significant codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’

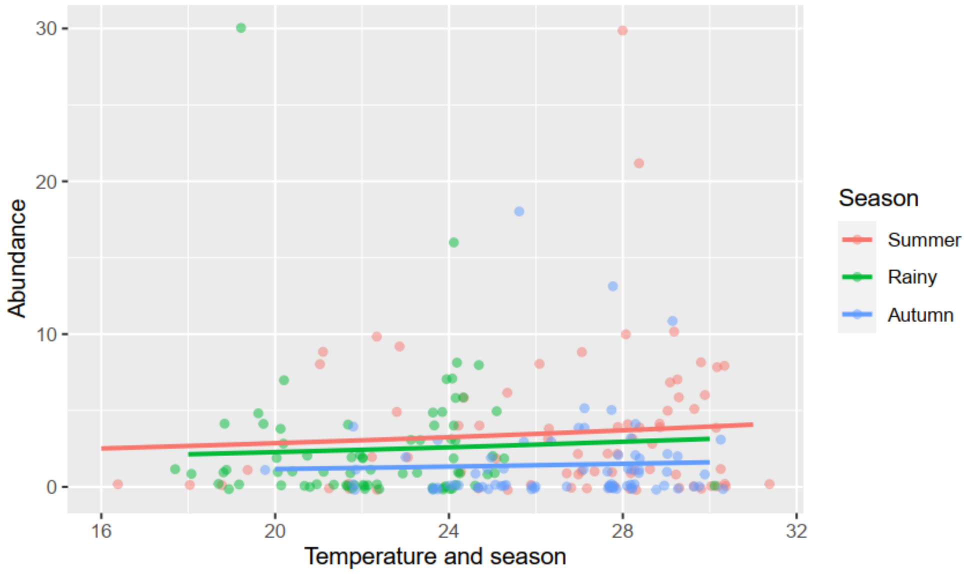

Figure 6: GLM showing relation of ladybird beetle abundance with temperature and season

4.4 Feeding efficiency of ladybird beetles of *C. septempunctata* and *P. luteopustulata* against aphids

The data indicated that *C. septempunctata* had overall high consumption rate (34.40 ± 1.67) as compared to *P. luteopustulata* (29.15 ± 1.84) i.e. number of prey consumed per day at room temperature i.e. 13.67±0.26°C and 65.30±0.69% RH showing relatively increase in feeding pattern with increase in temperature (Table 7).

Table 7: Mean number of aphids consumed

Temperature	Humidity	Mean number of aphids fed by	
		C. septempunctata	***P. luteopustulata***
11.72 ± 0.15°C	68.91 ± 0.96% RH	30.03 ± 1.42	23.52 ± 1.17
15.62 ± 0.25°C	61.70 ± 0.56% RH	38.77 ± 2.89	34.77 ± 3.28

5. DISSCUSSION

In this study, 17 species belonging to 11 genera from 4 tribes and subfamily Coccinellinae were recorded from Tribhuvan University premises with Shannon Diversity index (H) of 1.25 and evenness (J) of 0.44. Similarly, exclusive study in NARC, Khumaltar, Lalitpur found same 14 species which were same species except two species i.e. *Calvia quatuordecimguttata* and *Coelophora biplagiata* (K.C. et al. 2019).This may be due to the presence of both locations at a similar altitude within a range of 1300-1400masl.

The species diversity was comparatively higher in autumn and rainy than in summer in present study and high abundance in summer as compared to autumn and rainy. This is accordingly with Burgio et al. (2006) in which coccinellid populations showed peak in early summer and late summer. Again, Salehi et al. (2013) showed high abundance in June, decrease in abundance in summer again increase in September and October. Honek et al. (2015) studied that *C. septempunctata* and *H. axyridis* were frequent in the spring, became abundant in summer and maximum in late august. Elekcioglu (2020) also detected coccinellid populations high in spring and early summer then decreased. This may be because coccinellids display increased activity responding to aphid abundance during spring, seem to disappear in summer in spite of presence of aphids and reproduce in autumn to enter hibernation (Hagen 1962). In contrast, Maqbool et al. (2020) found high species diversity in summer with maximum abundance in August. Markovic et al. (2018) also showed an increase of coccinellid community diversity from beginning of spring to middle of summer then declination. This may be because the dominance of a species in the coccinellid community varies depending on the host plant (Vandereycken et al. 2013).

Diversity is highest in agricultural land followed by garden, planted forest and least in grassland in this study. This is supported by Finlayson et al. 2008 and Chakrabarti et al. 2012 in which the agro-ecosystem has higher species than the forest ecosystem. Previous study of coccinellid fauna in different landscapes of southern Michigan, U.S.A. also showed higher diversity in semi natural habitats such as grasslands and forests with variety of crops grown in smaller fields and lower diversity in large fields with annual crops (Woltz and Landis 2014). It may be because land cover diversity within landscape is positively related to natural enemy abundance (Isaia 2006).

The *C. septempunctata* associated with *M. rosae* was supported with the earlier reports of Omkar and Pervez 2004, Chakrabarti et al. 2012, Ahmad et al. 2017, Farooq et al. 2017, Ghani and Maalik 2020. *C. septempunctata* found with *R. maidis* was reported in Agarwala and Ghosh 1988, Omkar and Pervez 2002. *C. septempunctata* associated with *A. gossypii* is in accordance with Omkar and Pervez 2004, Conway and Kring 2010, Prabhakar and Roy 2010, Chaudhary and Singh 2012, Chakrabarti et al. 2012. The association of *P. luteopustulata* with *A. gossypii*, *O. sauzeti* with *Macrosiphoniella* spp. and *M. yomogifoliae*, has shown similarity with findings of Agarwala and Ghosh 1988, Chakrabarti et al. 2012. *M. rosae* as prey of *C. sexmaculata* is also in accordance with Agarwala and Ghosh 1988, Chaudhary and Singh 2012, Ahmad et al. 2017. The C. septempunctata was mostly encountered with several species as compared to other species. This is because *C. septempunctata* is generalist with a wide prey range which have helped in establishment in a wide range of areas (Omkar and Pervez 2002).

From the above result, temperature had a significant positive relation, and the probability of finding ladybirds is higher in summer. However, relation with humidity and precipitation is not compared due to errors. Bajia and Singh (2014) by correlation analysis showed that temperature had positive correlation and relative humidity had significant negative correlation with coccinellid of the mustard ecosystem. This finding is more or less similar to (Boda and Ilyas 2017) who reported that temperature had significant positive relation. These are in confirmation with Gurung et al. (2018) who observed non-significant positive relation with temperature and non-significant negative relation with rainfall. This may be because significant change in temperature and relative humidity does not affect coccinellids but greatly influences the aphid population. It seems that coccinellid population is mainly host dependent, it increases with host population (Bajia and Singh 2014).

The result showed that *C. septempunctata* was efficient in comparison to *P. luteopustulata*. Many similar observations showed conformity on consumption rate of coccinellids on aphids. Prabhakar and Roy (2010) recorded highest consumption by *C. septempunctata* (56±2.2) in comparision to *C. transversalis*, *C. sexmaculata*, *M. discolor* and *Pullus pyrocheilus*. 25±°C, 70±5% RH, 12hL: 12hD (Ali and Rizvi 2007). This work is also consonance with (Devi et al. 2010) *C. septempunctata* having highest feeding rate on *Toxoptera aurantii* among other predators viz. *C. septempunctata*, *C. transversalis*, *Oenopia sexareata* and *C. bissellata*. This is also in confirmation with (Maharjan et al. 2019) *C. septempunctata* was most efficient among *C. septempunctata* and *H. variegata*. This is largely attributed to differences in predator size, larger ones have relatively high voracity and increased energy requirements than smaller ones (Mishra et al. 2011, Mishra et al. 2012). Also due to difference in prey preference of these two species (Omkar and Mishra 2005c) and *C. septempunctata* being comparatively efficient exploiter of *L. erysimi* (Omkar and Srivastava 2003, Kumar et al. 2013).

6. CONCLUSION AND RECOMMENDATION

6.1 Conclusion

From this study, it can be concluded that species diversity in Tribhuvan University premises is found to be high in agricultural land. Species diversity of coccinellid is comparatively high in autumn followed by rainy and summer season even if abundance is higher in summer season. *C. septempunctata* is found to be associated with *M. pseudoartemisiae, M. yomogifoliae, R. maidis, A. gossypii,* and *M. rosae*. *C. sexmaculata, P. luteopustulata, O. sauzeti, and O. mimica* are found to be associated with *M. rosae, O. sauzeti* and *O. mimica* with *M. yomogifoliae,* and *P. luteopustulata* with *A. gossypii*. Temperature and season is found to have a significant effect on coccinellid abundance. *C. septempunctata* is potential predator of prey aphid *L. erysimi*.

6.2 Recommendations

- Since diversity is found to be high, surveys of ladybird beetles should be carried out in the entire country to provide an authentic database.
- *C. septempunctata* was found to be the potential biological control ladybird beetle, study can be carried out in large scale so that it can be used commercially in mass rearing for biological control.

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APPENDIX I: Species diversity of coccinellids

S.N	Scientific name	Total (ni)	Pi=ni/N	ln(pi)	Pi(lnpi)	[-Pi(lnpi)]
1	*C. sexmaculata*	11	0.0194	-3.94246	-0.07649	0.076485
2	*C. septempunctata*	407	0.717813	-0.33155	-0.23799	0.237988
3	*C. transversalis*	10	0.017637	-4.03777	-0.07121	0.071213
4	*C. bissellata*	12	0.021164	-3.85545	-0.0816	0.081597
5	*Coelophora* sp.	1	0.001764	-6.34036	-0.01118	0.011182
6	*H. sedecimnotata*	1	0.001764	-6.34036	-0.01118	0.011182
7	*H. kathmanduensis*	1	0.001764	-6.34036	-0.01118	0.011182
8	*H. variegata*	7	0.012346	-4.39445	-0.05425	0.054252
9	*J. quadrinotata*	2	0.003527	-5.64721	-0.01992	0.01992
10	*K. confusa*	36	0.063492	-2.75684	-0.17504	0.175037
11	*N. sexnotatus*	5	0.008818	-4.73092	-0.04172	0.041719
12	*O. kirbyi*	5	0.008818	-4.73092	-0.04172	0.041719
13	*O. mimica*	14	0.024691	-3.7013	-0.09139	0.09139
14	*O. quadripunctata*	1	0.001764	-6.34036	-0.01118	0.011182
15	*O. sauzeti*	16	0.028219	-3.56777	-0.10068	0.100678
16	*P. dissecta*	8	0.014109	-4.26092	-0.06012	0.060119
17	*P. luteopustulata*	30	0.05291	-2.93916	-0.15551	0.155511
		567				1.252358

APPENDIX II: Species diversity during summer season

S.N	Scientific name	Total(ni)	Pi=ni/N	ln(pi)	Pi(lnpi)	[-Pi(lnpi)]
1	*C. septempunctata*	220	0.814815	-0.20479	-0.16687	0.16687
2	*C. bissellata*	2	0.007407	-4.90527	-0.03634	0.036335
3	*Coelophora* sp.	1	0.003704	-5.59842	-0.02073	0.020735
4	*H. sedecimnotata*	1	0.003704	-5.59842	-0.02073	0.020735
5	*H. variegata*	6	0.022222	-3.80666	-0.08459	0.084592
6	*J. quadrinotata*	1	0.003704	-5.59842	-0.02073	0.020735
7	*O. kirbyi*	3	0.011111	-4.49981	-0.05	0.049998
8	*O. mimica*	8	0.02963	-3.51898	-0.10427	0.104266

9	*O. quadripunctata*	1	0.003704	-5.59842	-0.02073	0.020735
10	*O. sauzeti*	14	0.051852	-2.95936	-0.15345	0.153449
11	*P. luteopustulata*	10	0.037037	-3.29584	-0.12207	0.122068
12	*N. sexnotatus*	3	0.011111	-4.49981	-0.05	0.049998
		270				0.850515

APPENDIX III: Species diversity during rainy season

S.N	Scientific name	Total(ni)	Pi=ni/N	ln(pi)	Pi(lnpi)	[-Pi(lnpi)]
1	*C. sexmaculata*	4	0.036364	-3.31419	-0.12052	0.120516
2	*C. septempunctata*	85	0.772727	-0.25783	-0.19923	0.199232
3	*C. transversalis*	1	0.009091	-4.70048	-0.04273	0.042732
4	*C. bissellata*	6	0.054545	-2.90872	-0.15866	0.158658
5	*H. variegata*	1	0.009091	-4.70048	-0.04273	0.042732
6	*J. quadrinotata*	1	0.009091	-4.70048	-0.04273	0.042732
7	*O. kirbyi*	1	0.009091	-4.70048	-0.04273	0.042732
8	*O. mimica*	1	0.009091	-4.70048	-0.04273	0.042732
9	*O. sauzeti*	1	0.009091	-4.70048	-0.04273	0.042732
10	*P. luteopustulata*	8	0.072727	-2.62104	-0.19062	0.190621
11	*N. sexnotatus*	1	0.009091	-4.70048	-0.04273	0.042732
		110				0.968147

APPENDIX IV: Species diversity during autumn season

S.N	Scientific name	Total(ni)	Pi=ni/N	ln(pi)	Pi(lnpi)	[-Pi(lnpi)]
1	*C. sexmaculata*	7	0.037433	-3.2852	-0.12298	0.122975
2	*C. septempunctata*	102	0.545455	-0.60614	-0.33062	0.33062
3	*C. transversalis*	9	0.048128	-3.03388	-0.14602	0.146016
4	*C. bissellata*	4	0.02139	-3.84481	-0.08224	0.082242
5	*H. kathmanduensis*	1	0.005348	-5.23111	-0.02797	0.027974
6	*K. confusa*	36	0.192513	-1.64759	-0.31718	0.317183
7	*O. kirbyi*	1	0.005348	-5.23111	-0.02797	0.027974

8	*O. mimica*	5	0.026738	-3.62167	-0.09684	0.096836
9	*O. sauzeti*	1	0.005348	-5.23111	-0.02797	0.027974
10	*P. dissecta*	8	0.042781	-3.15167	-0.13483	0.134831
11	*P. luteopustulata*	12	0.064171	-2.7462	-0.17623	0.176227
12	*Novius sexnotatus*	1	0.005348	-5.23111	-0.02797	0.027974
		187				1.518825

APPENDIX V: Coccinellids population with weather parameters

Number	Temperature	Season	Number	Temperature	Season	Number	Temperature	Season
0	18	1	0	26	3	0	22	2
0	16	1	0	26	3	0	22	2
4	24	1	0	28	3	2	25	2
9	23	1	4	27	3	6	24	2
6	25	1	1	28	3	8	25	2
1	24	1	2	28	3	5	25	2
4	25	1	0	28	3	4	24	2
3	26	1	4	27	3	0	24	2
0	25	1	0	27	3	0	24	2
10	22	1	2	25	3	2	22	2
0	22	1	0	25	3	2	22	2
2	23	1	0	25	3	0	22	2
2	22	1	0	25	3	0	21	2
0	22	1	1	25	3	0	22	2
8	21	1	0	25	3	0	21	2
9	21	1	3	30	3	3	23	2
0	21	1	0	25	3	0	22	2
0	22	1	1	25	3	0	22	2
5	23	1	0	25	3	1	22	2
0	25	1	0	22	3	1	24	2
2	25	1	0	26	3	0	24	2
4	26	1	0	28	3	3	24	2
8	26	1	18	26	3	16	24	2
6	24	1	2	28	3	8	24	2
3	28	1	1	28	3	5	24	2
1	28	1	0	28	3	7	24	2
0	28	1	11	29	3	5	24	2
2	28	1	0	29	3	0	24	2
30	28	1	5	27	3	1	25	2

1	27	1	1	22	3	1	21	2
0	27	1	1	27	3	0	30	2
9	27	1	3	24	3	0	25	2
1	27	1	0	24	3	7	24	2
0	27	1	0	24	3	0	24	2
2	27	1	3	26	3	0	24	2
1	19	1	0	26	3	0	24	2
0	19	1	0	25	3	2	25	2
4	29	1	0	24	3	1	25	2
1	28	1	0	25	3	1	18	2
0	28	1	0	28	3	0	19	2
4	28	1	4	28	3	2	20	2
21	28	1	13	28	3	30	19	2
5	29	1	1	28	3	1	20	2
8	30	1	3	28	3	3	20	2
8	30	1	0	28	3	4	20	2
0	31	1	4	22	3	7	20	2
0	30	1	2	28	3	0	21	2
4	30	1	0	22	3	0	19	2
0	30	1	5	28	3	1	19	2
0	30	1	1	25	3	1	20	2
0	30	1	2	29	3	4	20	2
1	30	1	0	29	3	5	20	2
8	30	1	0	28	3	0	20	2
5	30	1	0	28	3	4	19	2
0	30	1	0	28	3	0	19	2
0	30	1	0	28	3	1	18	2
6	30	1	3	26	3	1	19	2
0	26	1	0	24	3	2	21	2
1	27	1	0	24	3	0	22	2
4	28	1	0	28	3	0	22	2
6	29	1	1	28	3	2	22	2
7	29	1	1	28	3	1	23	2
10	29	1	2	29	3	1	23	2
7	29	1	1	29	3	3	23	2
4	29	1	0	29	3	6	24	2
0	29	1	0	30	3	1	24	2
3	29	1	1	30	3	4	24	2
1	29	1	1	20	3	2	22	2
2	28	1	0	30	3	3	24	2
0	28	1	0	30	3	2	24	2
1	27	1	0	28	3	4	22	2

4	28	1	0	28	3	0	22	2
10	28	1	0	28	3	1	22	2
0	28	1	0	24	3	0	22	2
1	29	1	0	24	3	0	24	2
1	28	1	2	23	3	0	21	2

APPENDIX VI: Number of aphids consumed by ladybird beetles

Date	**Temperature**		**Humidity**		**Number of aphids fed by *Coccinella septempunctata***					**Number of aphids fed by *Propylea luteopustulata***				
	8am	**8pm**	**8am**	**8pm**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
29-Dec-19	10.6	10.7	62	60	16	11	10	7	37	12	18	13	3	16
30-Dec-19	10	10.9	70	54	26	28	22	25	10	29	40	44	65	22
31-Dec-19	10.2	11.9	62	55	37	70	32	38	73	29	13	35	12	25
1-Jan	9.5	11.9	63	60	21	4	16	38	52	24	26	17	26	20
2-Jan	10.9	12.6	69	66	3	5	17	32	37	5	36	46	0	26
3-Jan	11.1	11.3	71	75	17	50	79	11	14	58	20	26	33	30
4-Jan	10.9	11.4	77	79	10	50	43	16	14	22	5	21	27	27
5-Jan	10.4	11.4	75	77	27	47	41	46	16	41	34	31	4	7
6-Jan	9.7	10.8	70	72	21	28	8	15	17	23	10	5	27	27
7-Jan	10.9	10.9	74	72	12	15	69	22	54	5	36	5	29	20
8-Jan	10.8	11.4	89	79	14	20	72	16	17	41	5	0	17	20
9-Jan	10.8	10.8	81	81	10	52	29	41	44	5	5	25	14	20
10-Jan	10.5	11.7	74	77	16	22	0	32	65	14	65	50	11	42
11-Jan	11.1	11.4	77	75	30	27	5	37	68	28	18	20	18	22

12-Jan	10.8	12.8	75	70	18	19	78	65	32	15	35	26	18	42
13-Jan	10.9	12.9	80	69	12	44	40	8	0	16	40	14	0	20
14-Jan	12	13.4	73	74	22	22	16	22	16	26	8	56	2	9
15-Jan	12.7	14.8	70	67	36	19	0	27	88	7	30	35	29	25
16-Jan	12.9	13.2	67	71	22	19	65	53	62	47	28	30	28	24
17-Jan	13.1	13.1	74	75	33	29	18	79	24	38	58	53	24	32
18-Jan	12.4	14.2	80	69	21	54	44	40	61	27	11	37	20	3
19-Jan	11`.4	12.3	71	65	21	15	40	33	46	18	45	0	16	47
20-Jan	11	11.3	64	61	35	30	28	20	31	56	29	12	17	3
21-Jan	10.9	11	66	60	12	28	17	40	3	37	12	12	15	0
22-Jan	10.3	11.5	67	62	13	25	14	13	24	27	15	14	36	17
23-Jan	10.9	11.4	66	67	32	26	9	14	43	17	28	25	29	30
24-Jan	10.5	11.3	66	70	32	28	13	25	32	17	37	23	27	14
25-Jan	10.5	11.5	70	70	32	32	55	25	60	30	25	15	35	32
26-Jan	11.1	11.9	70	70	38	20	71	19	43	31	43	10	20	40
27-Jan	10.9	11.3	67	69	44	20	33	31	5	8	37	27	36	30
28-Jan	11.1	11.9	69	70	26	55	29	64	59	69	30	42	2	10
29-Jan	11.1	11.9	69	70	23	45	33	44	10	3	35	25	27	0
30-Jan	12.5	12.8	69	62	30	18	33	24	20	30	0	11	0	5
31-Jan	14	13.6	60	66	25	35	43	28	39	45	12	11	15	37
1-Feb	14.2	12.9	61	66	58	35	38	17	33	9	26	29	5	3
2-Feb	14.2	12.1	61	64	22	31	37	40	0	44	12	31	40	0
3-Feb	12	12.8	60	62	2	28	0	37	5	15	6	25	14	38

4-Feb	11.8	13	63	62	39	28	21	15	50	31	64	35	21	27
5-Feb	11.8	12.9	66	64	37	27	45	0	25	33	0	25	0	24
6-Feb	12.4	12.5	67	65	28	0	8	25	25	36	31	46	10	47
7-Feb	11.7	12.8	66	63	8	0	0	0	43	13	30	2	30	20
8-Feb	12.1	13.1	68	61	33	26	14	45	50	25	18	30	0	22
9-Feb	11.8	12.7	63	57	27	48	31	31	20	37	25	17	0	45
10-Feb	12.4	13.4	63	59	45	28	22	30	55	40	52	22	0	20
11-Feb	12.8	14.1	61	57	0	0	35	22	35	21	22	15	0	32
12-Feb	14.4	14.5	63	60	48	8	59	60	27	25	21	0	18	20
13-Feb	14.2	14.9	62	61	32	31	51	35	60	34	25	50	20	55
14-Feb	14.9	14.7	61	64	5	72	2	42	0	12	35	4	0	0
15-Feb	14.3	16.4	66	64	34	11	22	0	18	0	20	31	17	3
16-Feb	15.8	16.4	62	64	0	10	31	18	21	_	18	25	7	18
17-Feb	16.5	16.9	63	63	23	38	45	22	39	_	23	12	27	_
18-Feb	15.5	16.6	61	65	47	15	1	67	38	_	35	18	48	_
19-Feb	16.2	17	65	65	25	51	18	41	40	_	6	38	6	_
20-Feb	12.1	17.4	62	64	51	11	69	18	43	_	25	33	14	_
21-Feb	16.9	17.5	67	64	40	59	60	23	21	_	64	_	55	_
22-Feb	16.7	17.2	67	63	24	47	95	78	87	_	6	_	16	_
23-Feb	16.9	17.4	62	61	37	66	95	78	86	_	68	_	63	_
24-Feb	16.9	16.8	57	55	21	0	0	0	35	_	35	_	35	_
25-Feb	16.2	16.6	55	56	46	0	0	3	38	_	25	_	35	_
26-Feb	15.2	15.8	61	60	34	11	21	31	35	_	26	_	29	_

27-Feb	14.9	15.5	60	62	49	28	40	33	25	_	29	_	0	_
28-Feb	14.9	15.8	62	64	60	59	39	49	55	_	47	_	43	_
29-Feb	15.8	17.1	62	62	65	75	75	90	67	_	57	_	62	_
1-Mar	16.9	17.4	66	65	28	54	69	90	67	_	88	_	80	_
2-Mar	15.5	16.4	63	65	63	25	38	13	37	_	28	_	38	_
3-Mar	15	16.2	63	64	48	40	53	37	0	_	41	_	45	_
4-Mar	15.1	15.8	64	63	51	41	45	55	27	_	50	_	57	_
5-Mar	14.6	16	62	63	35	59	75	68	57	_	58	_	50	_
6-Mar	15.7	16.3	63	60	56	43	20	64	41	_	28	_	0	_
7-Mar	15.7	15.8	65	64	48	14	22	66	44	_	57	_	26	_
8-Mar	14.8	15.8	62	60	53	55	42	44	45	_	46	_	60	_
9-Mar	15.2	16.4	61	65	61	32	47	58	44	_	48	_	57	_
10-Mar	16.6	17.1	67	66	88	93	40	70	70	_	93	_	96	_
11-Mar	16.8	18.1	65	60	0	0	46	5	0	_	48	_	4	_
12-Mar	17.4	18.4	60	51	0	22	0	42	30	_	0	_	_	_
13-Mar	17.9	17.6	52	52	70	90	24	27	61	_	41	_	_	_
14-Mar	17.3	17.9	52	56	71	50	49	59	55	_	48	_	_	_
15-Mar	16.9	17.4	57	52	57	57	73	74	51	_	39	_	_	_

APPENDIX VII: Some photographs of the study

a, b, d: Field visit; c, e: Ladybird beetles on leaf; f: Aphids on plant; g: Counting aphids; h: Deposited ladybird beetle specimens