

1. INTRODUCTION

1.1. Background

There are about 300 species of clover of *Trifolium* genus in the Fabaceae family, found over the world (Kentish 2015). Among them *Trifolium repens* L., commonly called as White clover, Dutch clover, White Dutch clover, Shamrock, White trefoil, etc. is native to Africa, Europe, Western Asia, Russia and Pakistan (Kentish 2015). The centre of origin of *Trifolium repens* L. is the Mediterranean region (Sareen 2003) and the whole Europe while Central Asia, west of Lake Baikal as well as North Africa are indigenous area (Sareen 2003). *Trifolium* grows in different habitats: dry meadows, mud flats, wood margins, road sides, barren areas, plains, semi desert regions and mountains to the sub- alpine meadows (Sareen 2003). It also grows wild as a pasture plant in mountainous areas and is common on lawns and heavily grazed areas, on wastelands whereas it is cultivated as forage crops in different countries such as, China, India and Pakistan (Pratap 1997). Most of domesticated animals on which we depend for animal products like beef, mutton, poultry and dairy products depend on the insect pollinated legume plant like clover (Irshad and Stephen 2014). White clover usually is a perennial but behaves as an annual plant in warm and a biennial in cold climates (Duke 1981). White clover is used mostly for grazing, pastures hay, ground cover in horticultural situations and has a high nutritive value as it supplies a rich source of proteins and minerals making it suitable for grazing animals (Frame 2003). It blossoms from spring to early summer (Feb-June), all the flowers bloom from Feb-April and 50% of the plants are in bloom in May and June but few flowers are also seen in July (Pratap 1997). The flowers of white clover are small pea-shaped, white to pale pink in color and looks dense globular cluster standing at the top of erect peduncles. Each flower has five green or reddish fused sepals, five petals and ten tiny stamens and an ovary topped with a style and stigma (Sareen 2013).

1.2. Insect Pollinators of White clover

Pollination is an important stage in the sexual reproduction of flowering plants which involves the transfer of pollen from anther to the receptive stigma of flower. Pollinators play a key role in the maintenance of wild flower reproduction, the products of which support a wide range of invertebrates, birds and mammals (Wilson et al. 1999). Pollinators are also essential for food production and play the vital role in various plants including cultivated crops (Klein et al. 2007). Hence, there exists a strong relationship between

pollinators and flower of the plant that they pollinate (Pratap et al. 2012). Pollinators provide pollination services that are crucial for the productivity of agricultural and natural ecosystems. Over three quarters of the world's crops and over 80% of all flowering plants has been estimated to depend on animal pollinators especially bees (Mcgregor 1976). Over 75% of the world has been considered to depend on biotic pollinators (Prescott-Allen 1990). The extent of reliance of agriculture on animal pollinators is evaluated in 200 countries and recorded 70% of crops used globally for human consumption are dependent on animal pollinators (Klein et al. 2007). Cross fertilization in most of the agricultural and horticultural crops is brought about by physical agents: wind, water and gravity and biological agents: insects (chiefly the bees), birds and mammals (Kendall and Solomon 1971). Among insects, bees, flies, beetles, butterflies, moths, wasps and weevils are important pollinators of agricultural, horticultural and forage crops, ornamental and wild plants are all effectively pollinated by insects visiting flower for nectar or pollen (Pratap et al. 2012). White clover is not easily dispersed by wind due to its clove so, insect pollinators are needed to transfer its pollen (Duke 1981). Cross pollination between flowers is mostly done by insects and less efficiently by wind (Willmer and Stone 1989). The florets of white clover allow a wide variety of insects to access nectar (Woodcock 2012). White clover is very important legume species which provide a large forage source for pollinators: bees, butterflies, hoverflies, etc. Mostly long-tongued bees: bumble bees, honeybees, mason bees and cuckoo bees, bee flies, thick-headed flies, white butterflies and skippers visit clover flower for pollen or nectar (Hilty 2003) Certain wild bees, bumble bees and chiefly honey bees pollinate white clover (Oertel 1960). Honey bees are good pollinators due to presence of hairy body, colony strength, multiple and selective visits and they can also be transported to desired place either for migratory beekeeping or for pollination purposes in agricultural crops (Verma and Pratap 1993). Similarly, honey bees are most active at temperatures between 60° F (16° C) and 105° F (41° C) and wind above 15 miles per hour reduce their activity and stop it completely at about 25 miles per hour (Morse and Hooper 1985). Bees improve the efficiency of pollen deposits and its ability to visit several flowers enhances pollen tube access to ovules (Rodet et al. 1998). The efficiency of bees to deliver pollen from flower to flower depends on their behavior they adopt in foraging for nectar (Marshall et al. 1990). White clover allows desirable resources to honeybees.

1.3. Objectives of Study

The main objective of the study is to identify the pollinators of *Trifolium repens* L. and the specific objectives are as follows:-

1. To study the diversity of insect pollinators of *Trifolium repens* L.
2. To estimate relative abundance of pollinators.
3. To analyze the relation of insect pollinators with environmental variables (Temperature, Relative humidity and Light intensity).

1.4. Rationale

The white clover (*Trifolium repens* L.) has been selected for evaluating the different species of insect pollinators visiting it. It also grows wild as a pasture plant in mountainous areas and is common on lawns and heavily grazed areas, on wastelands. White clover is a very good source of nectar and pollen for *Apis cerena* and *Apis mellifera*. It plays important role in bee keeping as it provides good source of food for bees that put aid in honey production increasing economy of individual or group or whole nation. Since, it is not cultivated and grows on the wastelands in Nepal, less people know about its importance. And there is very few research es done in context of Nepal. Through this study, the important role of white clover and diversity of its insect pollinators will be evaluated and documented. Field study was selected at the premises of Bhaktapur Multiple Campus, Bhaktapur, the location was nearer and the ground has enough clover plant required for study.

1.5. Limitation

The study was confined only to the premises of Bhaktapur Multiple campus. The finding stated in this study is based on data obtained in the month of April during the flowering season. The study period was reduced to 26 days instead of one month due to continuous rainfall during the study period.

2. LITERATURE REVIEW

2.1. Biology of White clover pollen/ nectar and honey

White clover produces good amount of pollen and hence, it act as major sources of both nectar and pollen for honeybees. Its pollen grains are small (23.8 +/-1.04 mm) in diameter and brown in color. This plant provides food for cattle and also fixes atmospheric nitrogen and contributes to soil fertility, help in soil conservation, and prevent soil erosion. Mainly caterpillars use it as a food source and its foliage and seeds are eaten by birds. (Pratap 1997). The nectar selection is 0.05-0.40 mg/flower/day (Maurizio and Grafl 1982).

The honey made from white clover is bright in color (Howes, 1976) and has a characteristic mild and sweet flavor (Crane 1975). Its honey is acidic with pH-value of 4.0 (White *et al.* 1962 Good quantity of pure honey can be harvested from white cover.

2.2. Diversity of insects visiting the white clover flowers

Over 80% of total pollination is performed by insects in which nearly 80%is contributed only by bees (Thapa 2006). Mostly long-tongued bees: bumble bees, honeybees ,mason bees and cuckoo bees, bee flies, thick-headed flies, white butterflies and skippers visit clover flower for pollen or nectar (Hilty 2003) Certain wild bees, bumble bees and chiefly honey bees pollinate white clover (Oertel 1960). Hymenoptera are the most important natural pollinating agent, however, diptera (flies) and lepidoptera (butterflies and moths) may also visit flowers of white clover but are not effective pollinators (Harris 1987). Honeybees are considered to comprise 88% of all insect visitors to this crop (Goodman and Williams 1994). In Canada and Denmark *Megachile rotunda*, the alfa alfa leafcutter bee has seen to pollinate white clover efficiently (Williams 1991). Since, pollen and nectars of white clover come in contact with stigma (Oertel 1961), honeybees are attracted to white clover, hence, are thought to be effective pollinators (Free 1993).

Wild bees are apparently a negligible factor in the commercial pollination of white clover, however *Osmia*, *Halictus*, *Tetralonia* and *Bombus* are found fond of white clover flowers (Bohart 1960). Whereever white clovers are grown; bees are found visiting its flowers to some extent (Atwood 1943). Bumble bees and many other wild bees are also useful pollinators (Pedersen et al. 1961).

2.3. Foraging behavior of Honeybees

Researches done in pollination of apple, pear, plum, coconut and cherry flowers showed that *Apis mellifera* L. act as dominant species (Free 1970). *Apis cerana* can collect pollens up to 19mg (Punjabi et al. 1969). It starts to forage when it is two to three weeks old (Free 1970) reach peak pollen and nectar collection from January to March and peak honey storage from March to April (Naim and Phadke 1976). A honeybee can fly several kilometers to forage. It can travel up to 10 kilometres however, distance of 2.5 km has been recorded (Beekman and Ratnieks 2000). The forage area is smaller in presence of abundant nectar source (Williams 2001) as it has been found that 60.9% of flower heads of clover were visited by foraging honey bee within 10cm of the previously visited flower head and 13.6% were beyond 25m (Weaver 1965). A honeybee shows considerable area fidelity. It returns repeatedly to same small area of a field of flowers (Miller and Winn 1999). Bumblebees are found to fly about half as far between the white clover flower heads in nectar -poor area than foraging in nectar-rich area (Heinrich 1979). The direction and distance of pollinators is influenced by the amount of nectar in present in just visited flower. There was greater change in direction in a nectar-rich field of clover heads than in a nectar-poor depleted field but bees foraging on single flower does not exhibit such pattern whose reason is still unclear (Heinrich 1979).

2.4. Environmental variables affect activity of pollinators

Temperature, humidity, light intensity, solar radiation and wind speed are the main climatic variables that influence the activity of pollinators (Abrol 2010, Sarangi and Baral 2006). These variables are responded differently by different pollinators: foraging time and peak temperature of bumble bee is lower than that of honey bee (Zhao et al. 2011). Foraging time of bee depends upon environmental temperature. They forage from 11A.M.to noon and 2 to 3 P.M. when temperature is 21.5⁰C to 22.8⁰C (Verma and Chauhan 1985). In Romania, the number of *Apis mellifera* was high from 1000 to 1200 and 1400 to 1600 hour on lucerene crops. The maximum activity of *Apis mellifera* occurred between 19⁰C and 23⁰C and activity decreased conserably beyond this temperature (Nevkryta 1957). Further, peak activity was noticed between 21.5⁰C and 22.8⁰C when temperature (Ciurdarescu 1972). Dipterans were sufficiently active at low temperatures while honeybees and bumble bees were unable to forage (Wiering 1964). Diptera act as key pollinators in colder climatic

conditions (Gonzalez et al. 2009, Kearns 1992). Similarly, flies require shorter distances to travel for nectar and pollen as they shelter close to crops (Bennewicz 2011). Fungal gnats foraged even during foggy or rainy and cool weather conditions (Michael et al. 1980). Dipterans often depend on solar radiation to get heat for flight by perching either on ground or on flowers (Arroyo et al. 1982, Heinrich 1993).

Honeybees maintain constant thorax temperature of 33.7 °C –35.7 °C at air temperature between 10 °C to 27 °C (Kovac and Stabentheiner 2011). Bumble bees have well insulated large body mass and dense setae (Heinrich 1974) which allow them to retain heat to forage even in cold condition (Peat et al. 2005). Bumblebees can also maintain lower thorax temperatures (Morgan and Heinrich 1987), so, they can fly in lower temperature (Heinrich 1993).

3. MATERIALS AND METHODS

3.1. Study area: - Bhaktapur Multiple Campus premises

3.1.1. Location

Geographically, Bhaktapur is located in the eastern rim of Kathmandu Valley. It is about 13 km Far-East from Kathmandu, the capital of Nepal and also known as Bhadgaon of “The city of Devotees”. It lies between 85° 21” to 85 ° 32” E longitudes and 27° 36” to 27° 44” N latitude on Nepal. The attitude of the city ranges from 1372m to 2166m above the sea level with the area of 119 km². The study site - Bhaktapur Multiple Campus is located in Dudhpati-17, Bhaktapur Municipality which lies between 85°25’16.96”E longitude and 27°40’08.38”N latitude at the elevation of 4357 ft. It has perimeter 654.53 m and area 28982.93m² (Google Earth 2015). The campus covers the total area of 51 Ropni and 11 aana which is constructed in front of Bhajyapokhari (Bhaktapur Multiple Campus 2013).

3.1.2. Geomorphology

Bhaktapur district is divided into hill area and valley area and bounded by Manohara river and Durga Khola in North, Suryavinayak hill in south, Mahadev Pokhari in east and Manohara river in west (DDC 2059). The study area lies in valley area.

3.1.3. Climate

Bhaktapur has a humid sub-tropical climate. The average annual temperature ranges between 20°C-25°C. The maximum recorded temperature is 32°C and the minimum is 3°C. Average rainfall is 56mm and relative humidity is 95% (DDC 2059).



Map of Bhaktapur

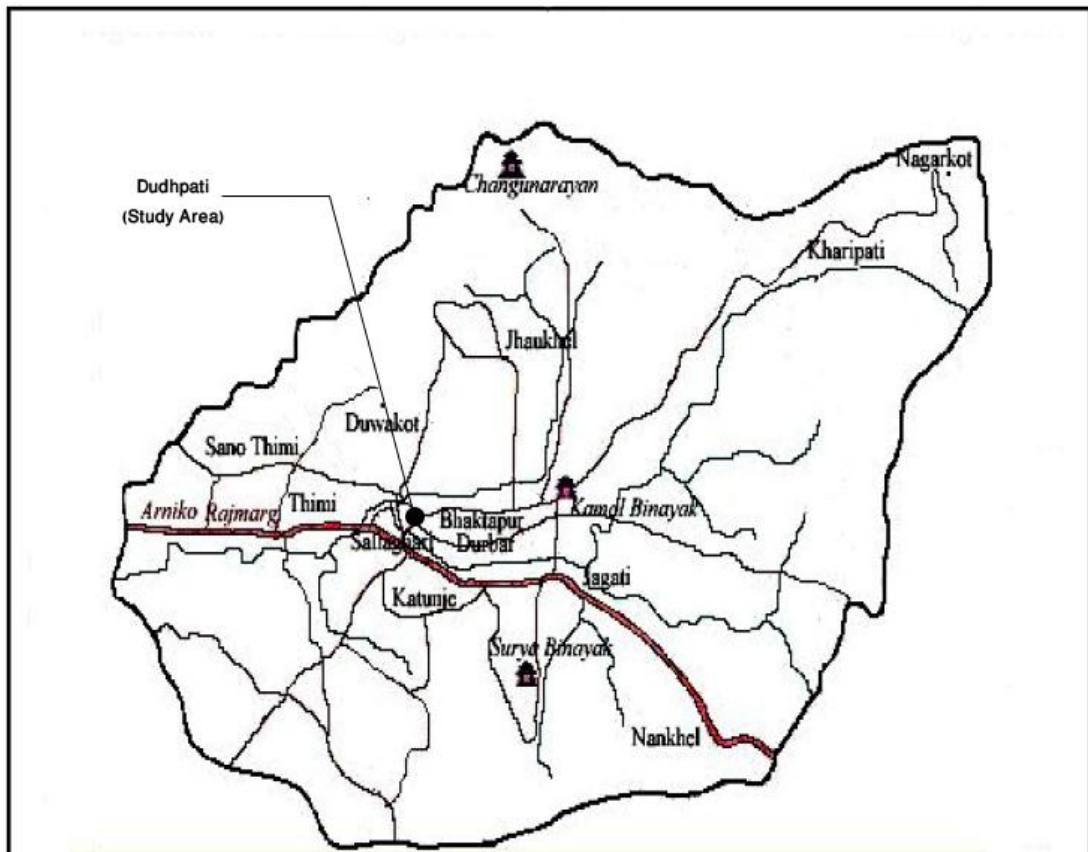


Figure 2.1: Map of Bhaktapur



Figure 2.2: Satellite view of Bhaktapur Multiple campus area showing research site

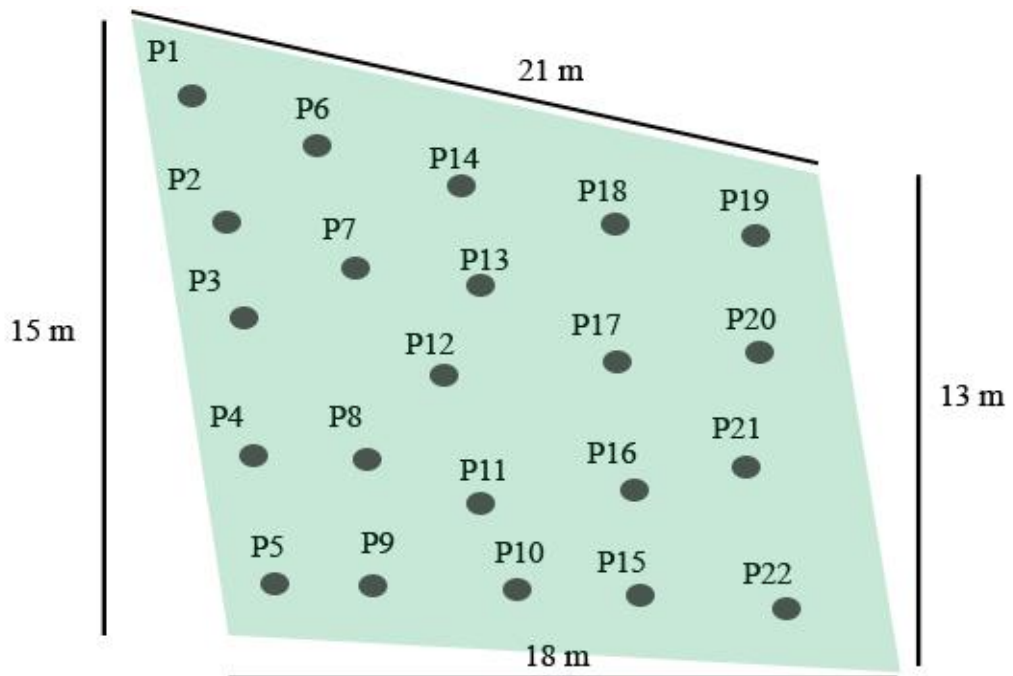


Figure 2.3: Plots used in field site to make quadrates

3.2. Field Survey

3.2.1. Reconnaissance Survey

The reconnaissance survey was done on March 2014 at the premises to observe whether flowers has commenced from the plants of white clover. Flowers already bloomed on March and the study was conducted from April 6 to April 27 in 22 plots.

3.2.2. Experimental Design and Procedure

Field experiment was conducted employing plots of size 10 x 10 ft² at different places of study area. Within each plot a quadrat of size 1 x 1 ft² was laid for sampling randomly. The insect pollinators and the specimens were collected from the ground of Bhaktapur Multiple Campus on April by applying random sampling method. Observation along with abundance of major insect pollinators was estimated in terms of their number visiting the flowers of clover plant for a period of 10 minutes.

3.2.3. Species collection and preservation techniques

The insect pollinators were collected with the help of sweep net of diameter 30.48cm (1 ft) with extendable handle at the time 9:00am to 10:00 am, 12:00 pm to 01:00 pm, 03:00 to 04:00 pm. The collected insect species were killed using killing bottle containing cotton soaked ethyl acetate. Killed insects were stretched in stretching board and finally they were preserved in the collecting box by pinning with the help of entomological pins of length (3 cm). Few naphthalene balls were put in the box as preservative.

3.2.4. Identification method

The entire collected specimen were identified with the help of various taxonomic literatures (Borror et al. 1981) and identification of butterflies were confirmed by Dr. Bhaiya Khanal at Natural History Museum, Swayambhu and other insect species by Mr. Ananda Shakya from National Agriculture Research Council (NARC), Khumaltar, Lalitpur, Nepal.

3.2.5. Species Deposition

The insect species collected during the field work will be deposited at the Central Department of Zoology, Tribhuvan University, Kirtipur, Nepal.

3.3. Data Analysis

3.3.1. Species Diversity

Species diversity was calculated by using Shannon's diversity index and community dominance by Simpsons index (Odum 1996), where:

$$\bar{H} = - \sum_{i=1}^k P_i \log P_i$$

Where,

\bar{H} = Shannon Index of diversity

P_i = Fraction of entire population made up of species $\left(\frac{n_i}{N}\right)$

R = no. of species

Σ = Sum of species i to species R

Note: High value of \bar{H} represents more diversified communities.

$$\text{Evenness (e)} = \frac{\bar{H}}{\log S}$$

Where, S = Total no. of species

Simpson's Index of Dominance or Community Dominance

$$C = \Sigma (P_i)^2$$

Where, $P_i = \frac{n_i}{N}$ and C = Simpson's Dominance Index

3.3.2 Statistical Analysis

Multivariate tests of species composition were carried out using a unimodal technique because of only presence/absence data (Leps and Smilauer 2003) and the gradient was short (1.5). Though, it used Canonical Correspondence Analysis (CCA) to show the relationship between insect species and environmental variables. The significance of the predictors was tested using a Monte Carlo permutation test. All tests were carried out using Canoco 5 (ter Braak and Smilauer 2012). For these data here used sampling plots and recording time as covariates and tested the effect of temperature, humidity and light intensity. Significant habitat characteristics were selected using a step-wise selection procedure.

Meteorological data of the study area during the study were collected from the device called Hobo Data Logger.

4. RESULTS

4.1. Insect pollinators in White clover

Various types of insect species which visited the flowers of white clover were collected and identified. A total number of 23 insect species belonging to 11 families and 3 orders were recorded. Out of these, 7 species belonged to Hymenoptera, 12 species to Lepidoptera and 4 to Diptera. They are listed in Table 4.1.

Table 4.1: List of Insect species visiting white clover flowers

Sr. No.	Insect species	Family	Order
1.	<i>A. cerana</i> Fabricius, 1793	Apidae	Hymenoptera
2.	<i>A. mellifera</i> Linnaeus, 1758	Apidae	Hymenoptera
3.	<i>Andrena</i> spp. Fabricius, 1775	Adrenidae	Hymenoptera
4.	<i>Polistes</i> spp. Latreile, 1802	Adrenidae	Hymenoptera
5.	<i>Vespa</i> spp. Linnaeus, 1758	Vespidae	Hymenoptera
6.	Ant	Formicidae	Hymenoptera
7.	Ichneumonid spp. Latreile, 1802	Ichneumonidae	Hymenoptera
8.	<i>Pieris brassicae</i> Linnaeus, 1758	Pieridae	Lepidoptera
9.	<i>Pieris candia</i> Sparrman, 1768	Pieridae	Lepidoptera
10.	<i>Colias erate</i> Esper, 1805	Pieridae	Lepidoptera
11.	<i>Colias fieldi</i> Ménétriés, 1855	Pieridae	Lepidoptera
12.	<i>Pontia daplidice</i> Linnaeus, 1758	Pieridae	Lepidoptera
13.	<i>Lampides boeticus</i> Linnaeus, 1758	Lycaenidae	Lepidoptera
14.	<i>Polyommatus semiargus</i> Rottemberg 1775	Lycaenidae	Lepidoptera
15.	<i>Precis almanac</i> Linnaeus, 1758	Nymphalidae	Lepidoptera
16.	<i>Aglais caschmirensis</i> Kollar, 1848	Nymphalidae	Lepidoptera
17.	<i>Helicoverpa armigera</i> Hübner, 1808	Noctuidae	Lepidoptera
18.	<i>Nyctemera adversata</i> Schaller, 1788	Arctiidae	Lepidoptera
19.	<i>Syntomoides</i> spp. Hampson 1893	Arctiidae	Lepidoptera
20.	<i>Eristalis cerealis</i> Latreille, 1804	Syrphidae	Diptera
21.	<i>Episyrphus Balteatus</i> De Geer, 1776	Syrphidae	Diptera
22.	<i>Musca domestica</i> Linnaeus, 1758	Muscidae	Diptera
23.	<i>Musca sorbens</i> Wiedmann, 1830	Muscidae	Diptera

Among these insects, *Apis cerana*, *Lampides boeticus*, *Andrena* spp., *Pieris brassicae*, *Colias fieldi* and *Apis mellifera* were found as frequent pollinators associated with White clover field while *Precis almona*, *Aglais caschmirensis*, *Polistes* spp., *Helicoverpa armigera* and Ichneumonid sp. were found visiting white clover less frequently.

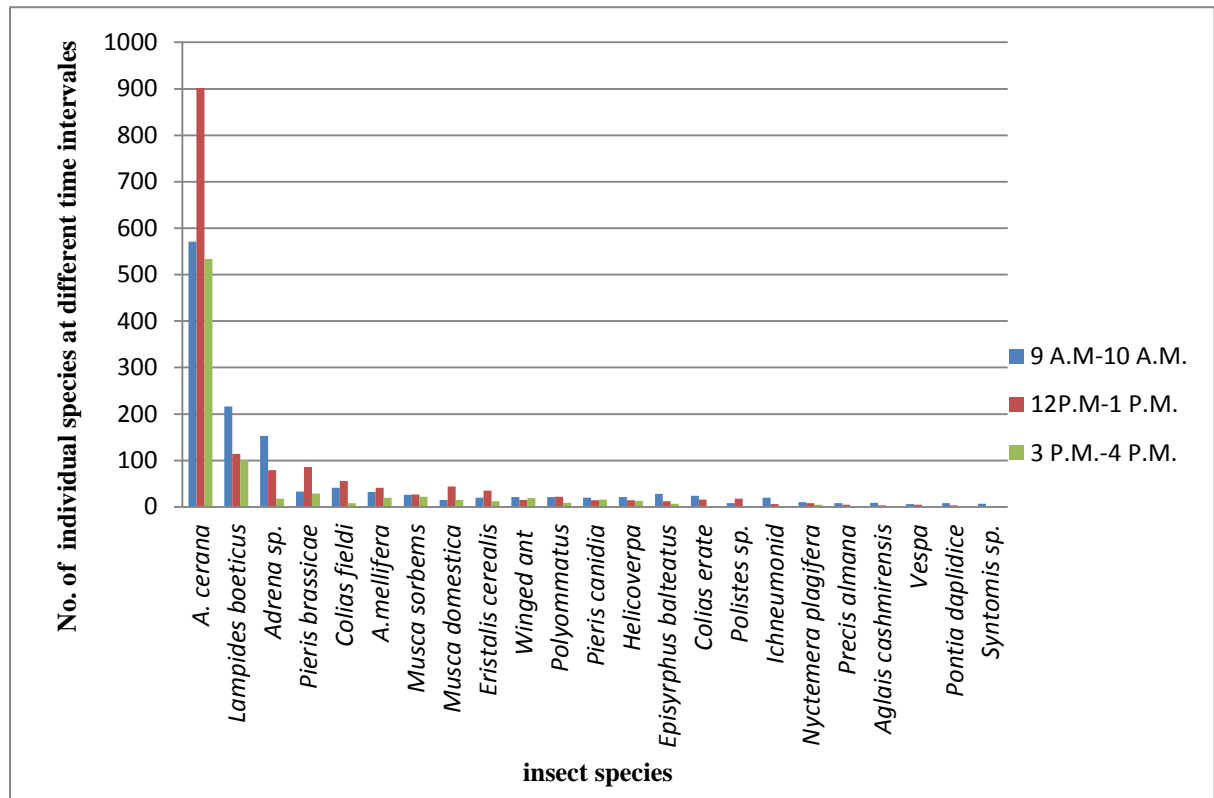


Figure 4.1.1: Graph showing average number of insect species that foraged at three different times; morning, day and evening respectively.

4.2. Species Diversity of pollinator associated with White clover plant

To determine the diversity for species of White clover, Shannon’s diversity index was applied. It was based upon the relationship between total number of species and individual species within a family.

The species diversity of white clover was recorded 0.80. Evenness recorded was 0.224. Community dominance within the species was found average 0.323. Among the species, *Apis cerana* and *Lampides boeticus* were found to be dominant as their value were 0.299 and 0.138 respectively. Similarly, *Syntomoides* spp. and *Pontia daplidice* species were found to be least dominant as their values were 0.0000027 and 0.000006 respectively.

4.3. Relative abundance of major insect pollinators

According to the study, the most abundant insect pollinators found in the white clover were Hymenopterans (68.71%). It was followed by Lepidoptera (23.24%) and Diptera (8.02%).

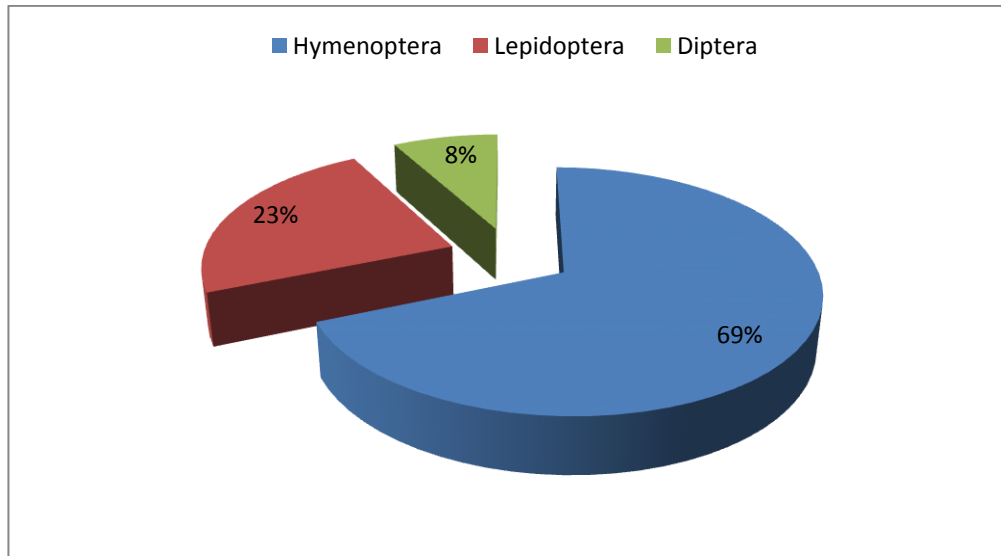


Fig 4.3.1: Abundance of different insect pollinators on White clover (*T. repens*)

Among all the insect species, *Apis cerana* were recorded the most (54.75%). It was followed by *Lampides boeticus* (11.72%), *Andrena* sps. (6.79%) and *Pieris brassicae* (4.02%).

4.4. Environmental variables

Environmental variables viz. Temperature, Humidity and Light intensity were recorded with the help of a device Hobo data logger. Temperature was measured in °F, relative humidity in % and light intensity in luminous/ft².

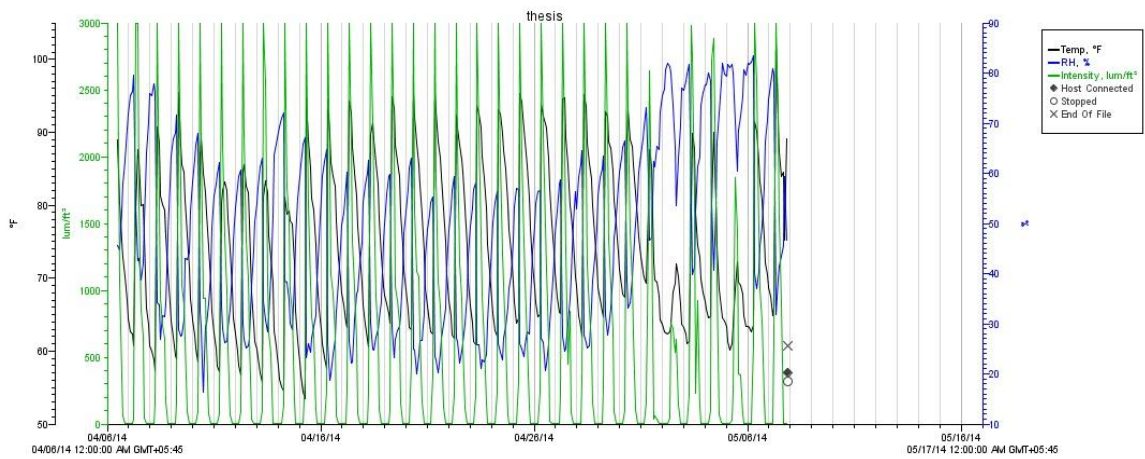


Figure 4.4.1: Graph of environmental data formed by Hobo data logger

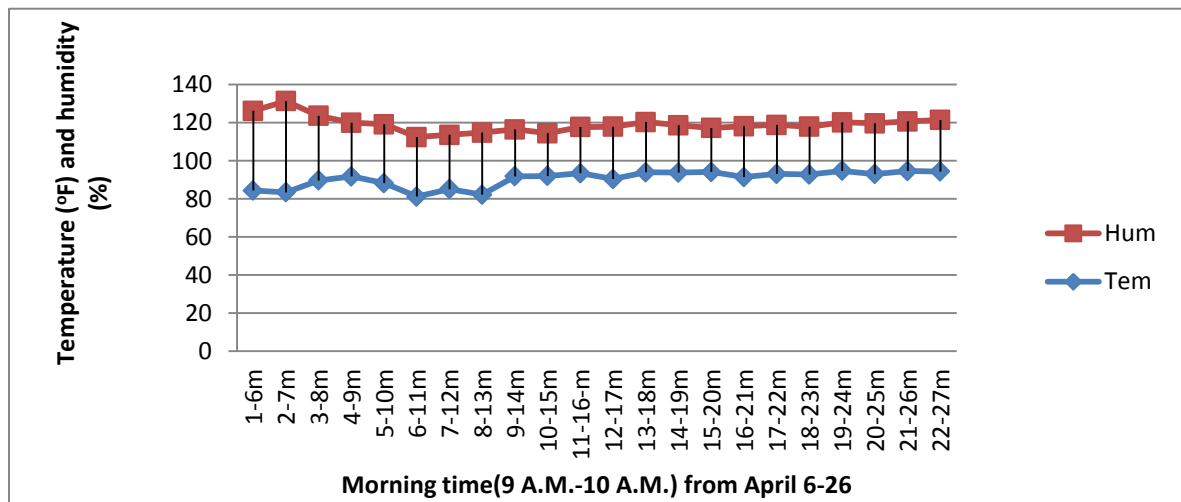


Figure 4.4.2: Graph of temperature and humidity on morning time from the day 1(April 6) to last day (April 26) of research.

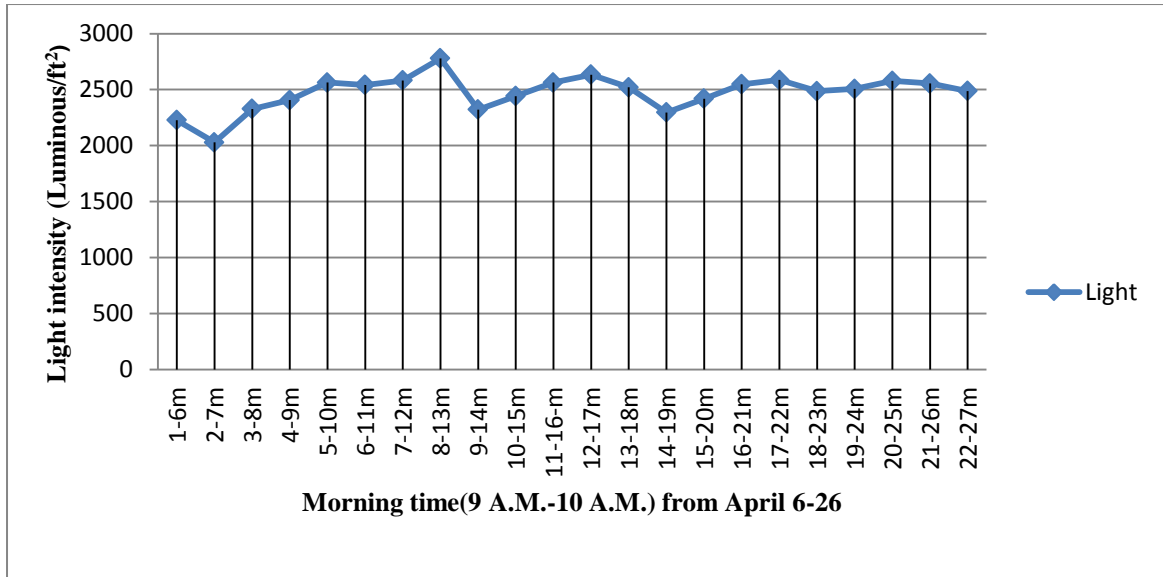


Figure 4.4.2: Graph of light intensity at morningtime from the day 1(April 6) to last day (April 26) of research.

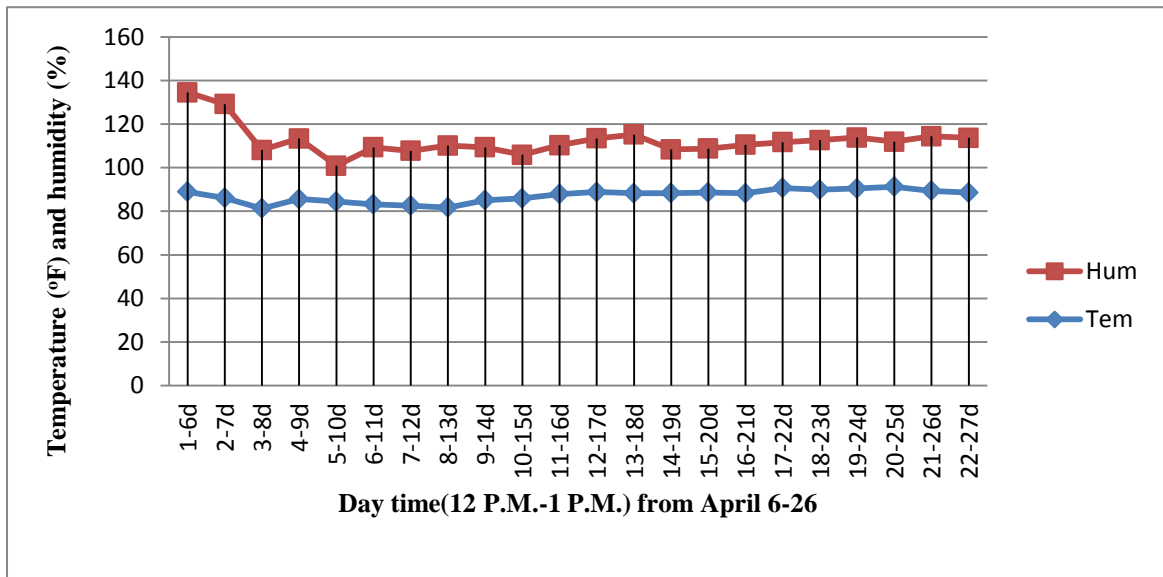


Figure 4.4.2: Graph of temperature and humidity on day time from the day 1(April 6) to last day (April 26) of research.

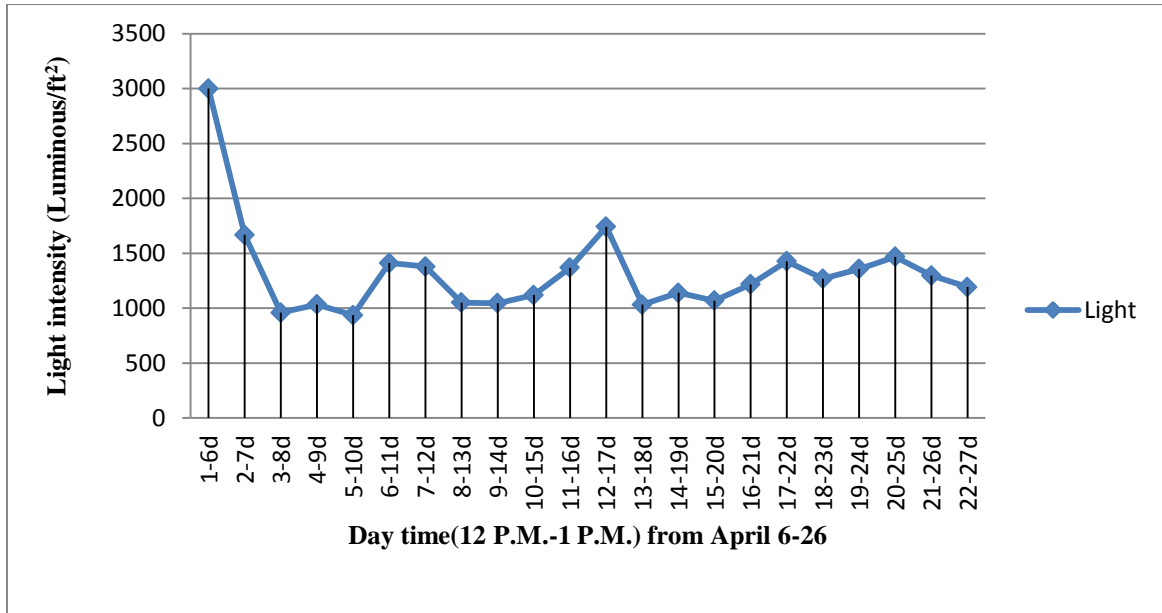


Figure 4.4.2: Graph of light intensity at day time from the day 1(April 6) to last day (April 26) of research.

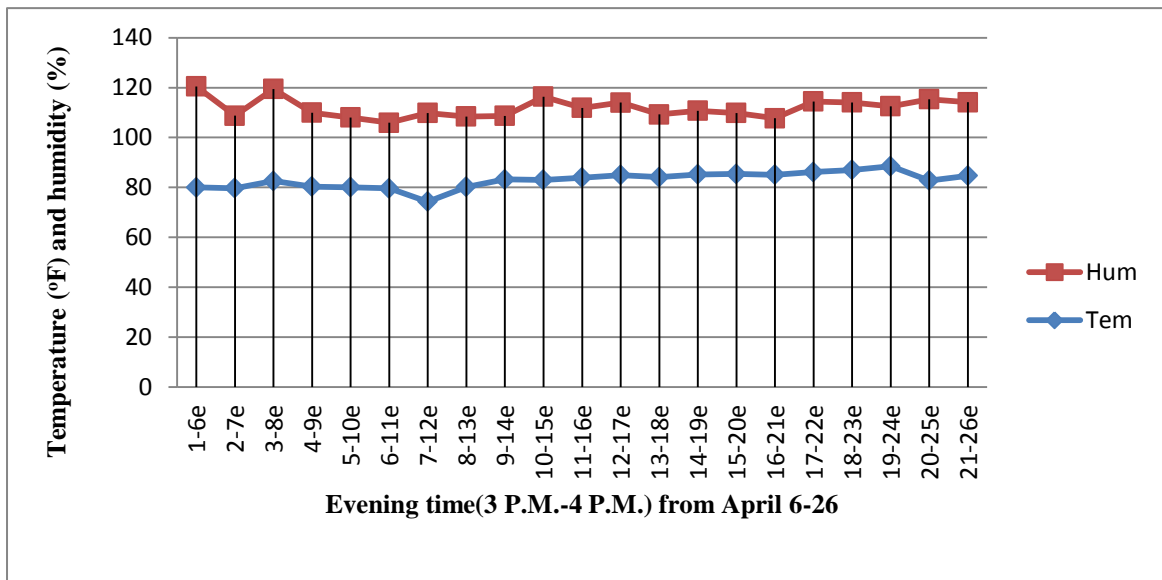


Figure 4.4.2: Graph of temperature and humidity on evening time from the day 1(April 6) to last day (April 26) of research.

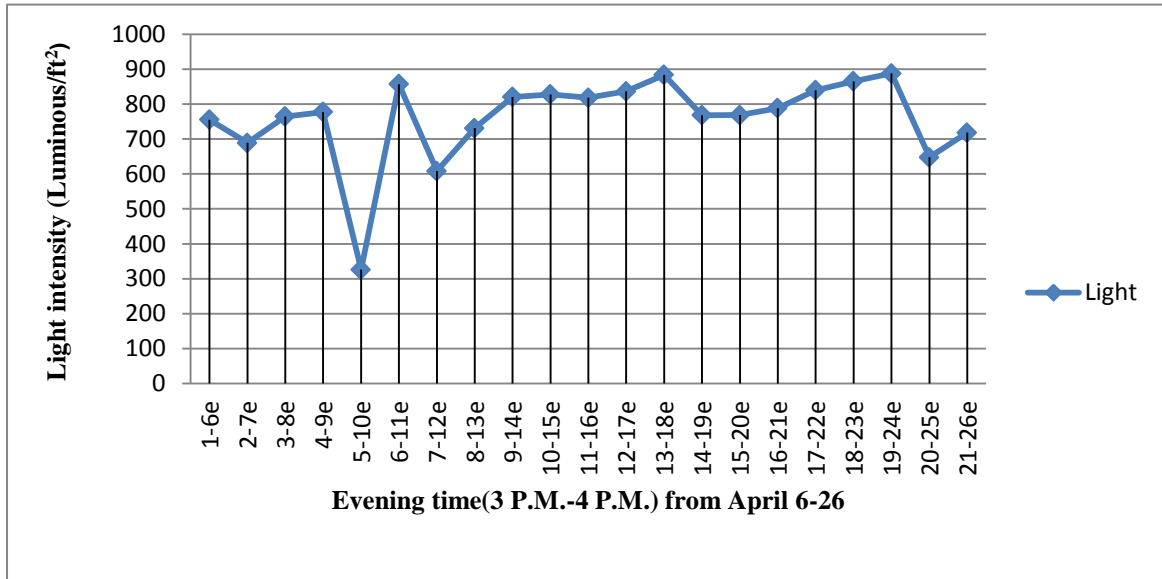


Figure 4.4.2: Graph of light intensity at evening time from the day 1(April 6) to last day (April 26) of research.

4.5. Correlation of insect species and environmental variables

The first CCA axis explains 10.2 and the second axis explain 6.11. The second axis explains the variables of the pollination data. Response data were compositional and have gradient 1.5 SD units long, so linear method was recommended, but unimodel one was used. Final model CCA forward selection was used to show the factor affecting pollinator visit in the *T. repens*. The total variation was 0.91747 and explanatory variables account for 19.1% (adjusted explained variation was 12.3%). Permutation test result on first axis was pseudo-F=5.2, P=0.002 and on all axis was pseudo-F=2.4, P=0.002. All together six environmental variables were used but among them five variables showed significant result during forward selection: Light (F=3.5, P=0.002), Temperature (F=3.8, P=0.002), Day (F=2.5, P=0.002), Evening (F=1.8, P=0.046) and relative humidity (F=1.8, P=0.028). Morning showed insignificant result during the forward selection test and removed for analysis (Table 4.5.1).

Table 4.5.1. The association between species and environmental variables

Name	Explains %	Contribution %	Pseudo-F	P-value
Light	5.3	26.4	3.5	0.002
Temperature	5.5	27.3	3.8	0.002
Day	3.5	17.4	2.5	0.002
Evening	2.4	12.1	1.8	0.046
Humidity	2.4	12.1	1.8	0.028

Light, humidity and temperature significantly affects the composition of pollinating species in white clover. The species that were recorded at the highest light intensity were *Syntomoides* spp., *Aglais caschmirensis*, *Colias erate*, *Precis almona* and *Episyrphus balteatus*. Similarly, high humidity was preferred by *Polistes* spp. and high temperature by *Colias fieldi*, *Apis mellifera*, *Pieris canidia*, *Musca domestica*, *Lampides boeticus*. And *Apis cerana* was abundance in all time and all condition (Fig. 4.5.2).

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.0761	0.0459	0.0305	0.014
Explained variation (cumulative)	8.29	13.29	16.61	18.13
Pseudo-canonical correlation	0.7431	0.7346	0.7334	0.5052
Explained fitted variation (cumulative)	43.34	69.46	86.82	94.79

Table 4.5.2: Summary table at the end of Canoco output

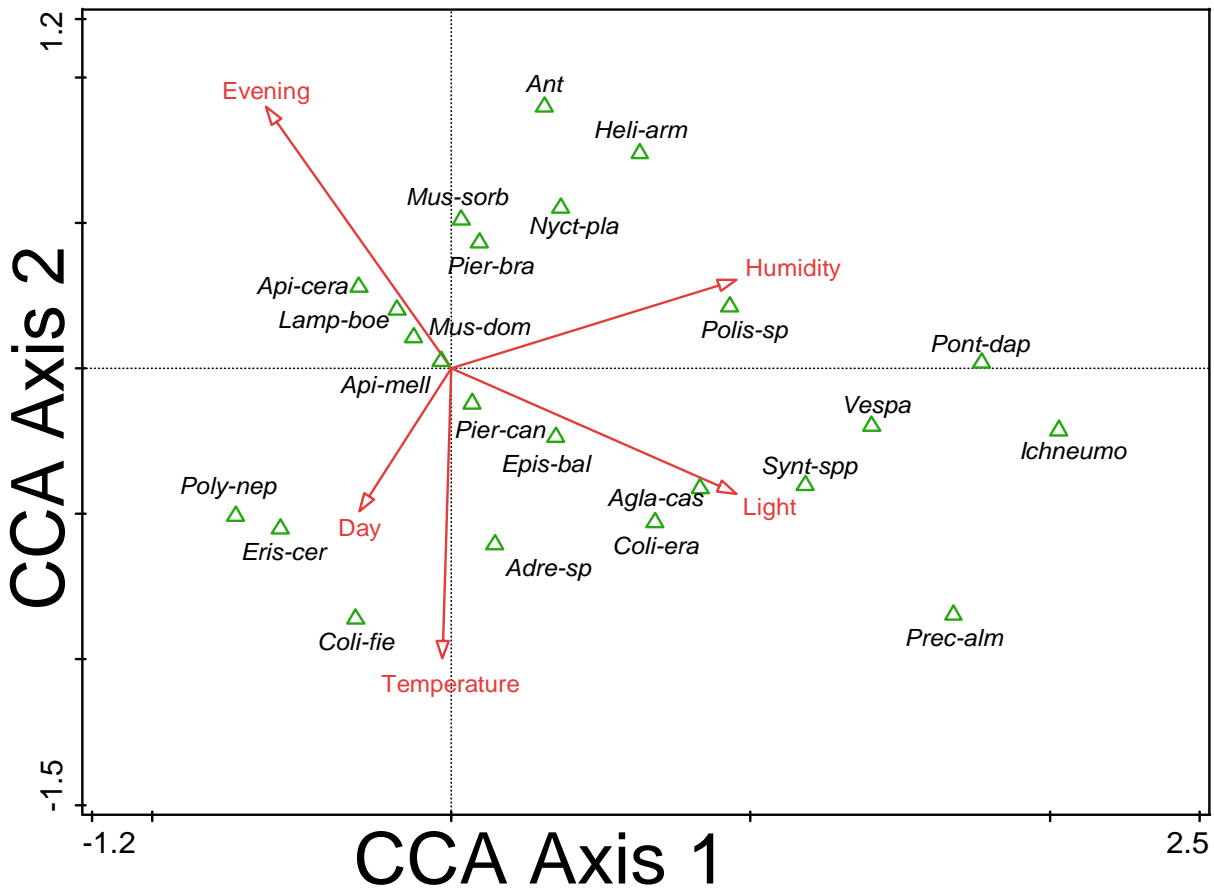


Fig.4.5.1: Relationship between the different environmental factors and distribution of pollinators. First axis explains 7.61% and second axis explains 4.59% of the total variation in the dataset.

5. DISCUSSION

The aim of this study was to find different types of insect pollinators, their abundance and associate them with the environmental variables: temperature, relative humidity and light intensity.

Insect pollinators play vital role for pollination of wild flower like *Trifolium repens* L. which grow on the waste lands. There are various insects belonging to different orders and families which forage on its flower for nectar and pollen. While foraging they help in cross-pollination which put an aid to increase in the density of plant.

The result presented in this study suggests that 23 different insect pollinators forage on the clover flower for nectar and pollen. They belong to three different orders: Hymenoptera, Lepidoptera and Diptera. Among them, the most abundant species observed on *Trifolium repens* L. was *Apis cerana* (54.75%) belonging to class Hymenoptera and family Apidae. *Apis cerana* was followed by *Lampides boeticus* (11.89%) belonging to Lepidoptera order, *Andrena* sp. (6.89%) of Hymenoptera order, *Polyommatus semiargus* (4.08%), *Colias fieldi* (2.90%) of Lepidoptera order and *Apis mellifera* (2.56%) of Hymenoptera order.

Similarly, the least found insect species pollinating clover flower were *Polistes* sp. (0.77%) Ichneumonid sp.(0.72%), *Nyctimera adversata* (0.63%), *Aglais caschmirensis* (0.36%), *Vespa* sp. (0.33%), *Pontia daplidice* (0.30%) and *Syntomoides* sp. (0.25%). This finding is in contrast to the finding of Potts et al. 2001, who reported bumble bees as the excellent pollinator of clover among 90% of bees belonging to Hymenoptera order in Mt. Carmel.. Such contradicting result is due to location of Mt. Carmel at high altitude as bumble bees prefer plants at high altitude (Potts et al. 2001) and they can reach the nectar more easily (Sarah et al. 1991). In the study area, *Apis cerana* has been found to be the most abundant pollinators, being native species and they are attracted by the flowers of *Trifolium repens* L. for nectar and pollen required for them. Similarly, such comparatively large mass of clover field is not found near the study area, so bees may be attracted towards the clover field. However, the number of bees counted is less because white clover pastures do not cover the study area completely. Allowance must be made for land taken up by buildings, football ground, basketball court, stage, non- flowering crops and trees. Hence, three-fourths of the total areas would be unattractive to bees.

A study done in the insect pollinators in Chitwan measured honeybees as the main pollinators and other bees were also recorded as the visitors of white clover (Thapa 2006),

which is similar to the research includes maximum number of honeybees i.e. *Apis cerana* and *Apis mellifera* and other species like *Andrena* spp., *Vespa* spp, Ichneumonid spp. ant and *Polistes* spp. *Trifolium repens* L., cross pollinated plant species is pollinated by wide range of insect pollinators, chiefly *Apis mellifera* L. and *Bombus* sp. (Roy and Burdon, 1983). White clover flowers are strongly adapted to bee pollination; hence bees which belong to family Apidae are mostly attracted for nectar (Kevan and Baker 1983). White clover species are insectary and pollinator friendly crops (Vaughan and Madar, 2008). In the study done by Rodet in 1997 explained flowers of white clover as hermaphrodite and self- compatible and pollinated particularly by bees belonging to the family Apidae. *Andrena* species and *Apis mellifera* visited clovers including both red and white clovers in New Heaven (Stoner, 2011). Hence, white clover being white is particularly attractive to the bees.

Butterflies of different kinds, being diurnal are also found during the study, it may be due to presence of various other colored flowers in the garden as well as wild condition. Among butterflies, *Lampides boeticus* were found frequently visiting the clover flowers as butterflies and skippers also act as pollinators of clover flower (Anon 2013). While some moths like *Nyctemera adversata* and *Syntomoides* sps. were also found hovering as they often visit the whitish to yellowish, strongly scented flower like clover which open nocturnally (Kevan and Baker 1983). Moths are mostly active at night and are attracted by scent and the contrasting white on background of dark foliage even from the distance (Kevan and Baker 1983). Moths are mostly nocturnal and prefer white flowers (Orville 2016) and the colour of flower of white clover is white.

Polistes species were also observed since there were many hives built on the college buildings near the study field. Diptera possess lapping mouthparts, are considered as primitive pollinators (Kevan and Baker 1983). Ant belonging to the family formicidae was also collected during the study; however its role in pollination is not fully understood (Kevan and Baker 1983), however its physical movement on the flower might fascinated easy pollination. Furthermore, parasitic hymenoptera like Ichneumonid sps. visited at the daytime for nectar of white flowers of clover (Kevan and Baker 1983).

The insect pollinators are greatly influenced by various environmental variables; wind, light intensity, humidity, temperature, etc. The key climatic variables, temperature, humidity, light intensity, solar radiation and wind speed are correlated with activity of pollinating

species (Abrol, 2010). Among the environmental factors, temperature is the most important factor that effect activities of many insects and it depends on the species. The insects like butterflies are active enough as pollinators only when the temperature of the environment is high which make them easy to fly by making their wing muscles warm (Sarah et al. 1991). The study also showed that butterflies like *Colias fieldi*, *Pieris canidia*, *Lampides boeticus*, etc. were active during high temperature on the clover field. Similarly, honey bees prefer to forage at higher temperature i.e. between 15°C and 40°C (Anon 2013). In the clover field, *Apis cerana* was found foraging most of the time irrespective of temperature change, however its population was higher at warm temperature. Hence, pollination is influenced by temperature (Bula and Massengale 1972).

Very least number of dipteran flies particularly hoverflies were recorded in the study which is similar to the study of Jonathan et al. 2014 who found fewer hoverflies in the white clover crop in Central Kentucky, USA.

Pollination is also affected by air humidity (Staszkeski, 1996) and *Polistes* spp. was found to be highly influenced by high humidity. In the study of Peat and Goulson, 2005 average temperature and relative humidity were recorded between 1200 and 1300 hours when insect activities were observed high. Here activity of butterflies was high at 50%-60% humidity when nectar secretion is high. In the study on white clover flower also most of the insect pollinators were active between 12:00- 01:00 when the temperature ranges between 28°C-31°C and relative humidity between 20.98%-43%. *A. cerana* were the most abundant when temperature ranges from 28°C-33°C.

Diptera often find shelter near cover crops (Bennewicz, 2011) and act as key pollinators in colder regions (Gonzalez et al., 2009), hence dipteran species were recorded in the study.

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

During the study period of about a month from 6th April 2014 to 27th April 2014, total 23 different insect species were collected and identified. Out of them, 7 species belonged to Hymenoptera, 12 to Lepidoptera and 4 to Diptera.

Among different insect species belonging to various families, *Apis cerana* constituted as the most abundant insect pollinator belonging to Hymenoptera order and Apidae family. *A. cerana* being native honey bee species and also the study area being located near forest on south direction made its number high on the pollination of white clover.

The result of the study shows the importance of honeybee as the pollinator. Various types of butterflies, dipteran flies like houseflies, syrphid flies, etc were also observed pollinating white clover flowers. Most of the pollinators were found to feed nectar and pollen of the clover flower which indicates clover plant as the important foraging vegetation.

The insect pollinators were found greatly influenced by the environmental factors like temperature, relative humidity and light intensity. The foraging activities of honeybees depend on the temperature and humidity mostly.

6.2. Recommendations

Based on this research, following are important recommendations:-

- ❖ The clover flowers are the important foraging plants for most of the insects pollinators, so its plantation near crop plants may increase the crop production as these insects also help in pollination of cultivated plants.
- ❖ The clover plant is also preferred by cattle, so it is important for grazing domestic cattle. Furthermore, clover plants were found to collect by local farmers for their domesticated cattle as fodder. According to them, it increases milk production.
- ❖ Honey produced by bees through clover pollen has great medicinal value such as it is effective for high blood pressure, healing wounds, etc so its commercial plantation and bee keeping must be encouraged for increasing the economy of local people.

- ❖ The people must be aware about the importance of clover and its growth on the wastelands should be utilized for bee keeping.

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Plate 4.1: Photographs of the selected pollinators prevalent in the white clover field

	
<p>1. <i>Pieris canidia</i> Sparman, 1768</p>	<p>2. <i>Pieris brassicae</i> Linnaeus, 1758</p>
	
<p>3. <i>Colias erate</i> (Male) Esper, 1805</p>	<p>4. <i>Colias erate</i> (Female) Esper, 1805</p>
	
<p>5. <i>Colias fieldi</i> Ménétriés, 1855</p>	<p>6. <i>Pontia daplidice</i> Linnaeus, 1758</p>



7. *Aglais caschmirensis* Kollar, 1848



8. *Precis almana* Linnaeus, 1758



9. *Lampides boeticus* Linnaeus, 1767



10. *Polyommatus semiargus*
Rottemburg, 1775



11. *Syntomoides* spp. Hampson, 1893



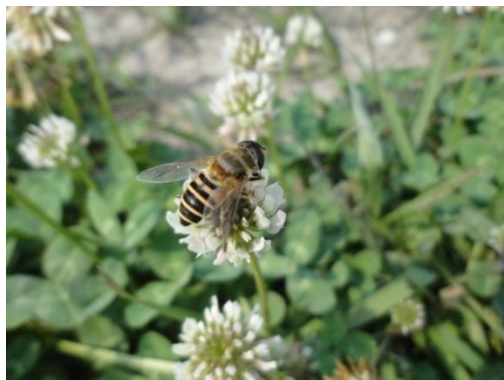
12. *Helicoverpa armigera* Hübner,
1808



13. *Nyctemera adversata* Schaller, 1788



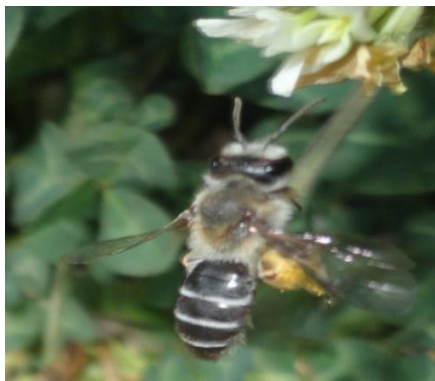
14. *Polistes* spp, Latreille, 1802



15. *Apis cerana* Fabricius, 1793



16. *Apis mellifera* Linnaeus, 1758



17. *Andrena* spp. Fabricius, 1775



18. *Vespa* Linnaeus, 1758



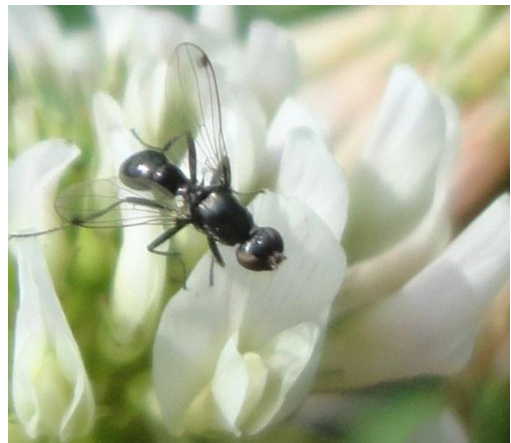
19. *Episyrrhus balteatus* De Geer, 1776



20. *Eristalis cerealis* Latreille, 1804



21. Ichneumonid spp. Latreille, 1802



22. Ant



23. *Musca domestica* Linnaeus, 1758



24. *Musca sorbens* Wiedmann, 1830

Plate 2: White clover field



i. White clover field



ii. White clover field



iii. Hobo data logger



iv. Researcher collecting insect



v. Researcher counting insect pollinators



vi. Quadrant thrown in the clover field

APPENDIX

Table 1: Shannon's Index for Species Diversity of White clover

Species	PilogPi	Evenness(e)= $\frac{\bar{H}}{\log S}$	(P _i) ²	
<i>Apis cerana</i>	-0.143	0.226	0.2998	
<i>Apis mellifera</i>	-0.04		0.0006	
<i>Andrena</i> spp..	-0.08		0.0047	
<i>Lampides boeticus</i>	-0.109		0.0138	
<i>Vespa</i> spp.	-0.034		0.0408	
<i>Pieris canidia</i>	-0.026		0.0002	
<i>Colias erate</i>	-0.022		0.0001	
<i>Colias fieldi</i>	-0.025		0.0002	
<i>Helicoverpa armigera</i>	-0.08		0.00001	
<i>Precis almana</i>	-0.009		0.00001	
<i>Polistes</i> spp..	-0.009		0.00001	
<i>Polyommatus semiargus</i>	-0.056		0.0016	
<i>Pieris brassicae</i>	-0.025		0.0002	
<i>Nyctemera adversata</i>	-0.014		0.00004	
<i>Syntomoides</i> spp.	-0.006		0.00001	
<i>Pontia daplidice</i>	-0.008		0.000009	
<i>Aglais caschmirensis</i>	-0.009		0.00001	
<i>Musca domestica</i>	-0.022		0.0008	
<i>Eristalis cerealis</i>	-0.032		0.0003	
<i>Episyrphus balteatus</i>	-0.024		0.0002	
<i>Ichneumonid</i> spp.	-0.015		0.00005	
<i>Musca sorbens</i>	-0.035		0.0004	
Unidentified spp.	-0.027		0.0002	
Total no. of species=3666	$\bar{H} = -\sum_{i=1}^k P_i \log P_i = 0.806$			$C = \sum (P_i)^2 = 0.364$

Table 2: List of insects visiting clover flower at different time intervals

	9 A.M-10 A.M.	12P.M-1 P.M.	3 P.M.-4 P.M.
<i>A. cerana</i>	571	902	534
<i>Lampides boeticus</i>	216	114	101
<i>Adrena</i> spp.	153	79	18
<i>Pieris brassicae</i>	33	86	29
<i>Colias fieldi</i>	41	56	8
<i>A.mellifera</i>	32	41	20
<i>Musca sorbems</i>	26	27	22
<i>Musca domestica</i>	15	44	15
<i>Eristalis cerealis</i>	20	35	12
Winged ant	21	15	19
<i>Polyommatus</i>	21	22	9
<i>Pieris canidia</i>	20	14	16
<i>Helicoverpa</i>	21	14	13
<i>Episyrphus balteatus</i>	28	12	7
<i>Colias erate</i>	24	16	2
<i>Polistes</i> spp.	8	18	2
Ichneumonid spp.	20	6	0
<i>Nyctemera plagifera</i>	10	8	5
<i>Precis almana</i>	8	5	0
<i>Aglais cashmirensis</i>	9	3	1
<i>Vespa</i> spp.	6	5	1
<i>Pontia daplidice</i>	8	3	0
<i>Syntomoides</i> spp.	7	2	0
Temperature(°F)	85.84	86.97	86.87
Relative humidity(%)	27.50	25.36	30.26
Light intensity(lum./ft ²)	2348.99	1332.76	883.60

Table 3: Environmental data and species abundance

Date and time	Temp.	Humidity	Light	Morning	Day	Evening	Pl-Abun
1-6m	84.364	41.871	2226.5	1	0	0	76
1-6d	88.902	45.614	2998.9	0	1	0	102
1-6e	80.05	40.435	755.15	0	0	1	46
2-7m	83.462	47.916	2028.1	1	0	0	96
2-7d	86.119	43.001	1667.4	0	1	0	88
2-7e	79.7	29.067	688.85	0	0	1	40
3-8m	89.665	33.979	2327.25	1	0	0	52
3-8d	81.244	26.782	959.1	0	1	0	55
3-8e	82.605	36.875	764.65	0	0	1	40
4-9m	91.795	28.225	2405.25	1	0	0	50
4-9d	85.577	27.76	1033.8	0	1	0	68
4-9e	80.364	29.604	777.45	0	0	1	34
5-10m	88.286	30.879	2562.75	1	0	0	54
5-10d	84.407	16.439	936.4	0	1	0	96
5-10e	80.125	27.919	3265.35	0	0	1	42
6-11m	81.157	31.318	2542.25	1	0	0	32
6-11d	83.199	26.136	1410.3	0	1	0	73
6-11e	79.642	26.285	856.95	0	0	1	35
7-12m	85.15	28.46	2581.8	1	0	0	43
7-12d	82.575	25.09	1378.8	0	1	0	96
7-12e	74.297	35.526	608.25	0	0	1	23
8-13m	82.222	32.61	2779.15	1	0	0	33
8-13d	81.73	28.391	1051.4	0	1	0	41
8-13e	80.215	28.213	731	0	0	1	17
9-14m	91.898	24.629	2321.4	1	0	0	90
9-14d	85.082	24.233	1044.8	0	1	0	79
9-14e	83.267	25.403	820.35	0	0	1	21
10-15m	92.005	22.502	2442.25	1	0	0	78
10-15d	85.849	20.027	1120.2	0	1	0	66
10-15e	83.047	33.355	827.65	0	0	1	49
11-16-m	93.472	24.264	2563.85	1	0	0	59
11-16d	87.894	22.337	1369.3	0	1	0	30
11-16e	83.917	27.99	818.15	0	0	1	32
12-17m	90.474	27.438	2633.05	1	0	0	76
12-17d	88.81	24.632	1741.3	0	1	0	52
12-17e	84.949	29.052	836.45	0	0	1	59
13-18m	93.993	26.388	2520.65	1	0	0	84
13-18d	88.259	26.925	1031.6	0	1	0	53
13-18e	84.165	25.13	883.3	0	0	1	54

14-19m	93.837	24.862	2296.1	1	0	0	62
14-19d	88.334	20.04	1140	0	1	0	73
14-19e	85.198	25.538	768.3	0	0	1	56
15-20m	94.141	23.121	2418.1	1	0	0	54
15-20d	88.626	20.119	1069	0	1	0	77
15-20e	85.399	24.418	768.35	0	0	1	40
16-21m	91.428	26.78	2547.75	1	0	0	57
16-21d	88.259	22.201	1217.7	0	1	0	39
16-21e	85.087	22.609	788.45	0	0	1	39
17-22m	93.117	25.704	2586.2	1	0	0	54
17-22d	90.657	20.975	1426.4	0	1	0	86
17-22e	86.192	28.253	839.35	0	0	1	24
18-23m	92.81	25.118	2485.85	1	0	0	43
18-23d	89.87	22.706	1268.9	0	1	0	53
18-23e	87.034	27.051	865.35	0	0	1	49
19-24m	94.707	25.452	2506.65	1	0	0	66
19-24d	90.424	23.396	1356.8	0	1	0	68
19-24e	88.524	24.034	887.7	0	0	1	42
20-25m	93.024	26.679	2578.1	1	0	0	60
20-25d	91.216	20.655	1468.4	0	1	0	82
20-25e	82.793	32.573	647.45	0	0	1	56
21-26m	94.634	26.019	2553.95	1	0	0	54
21-26d	89.316	25.058	1297.5	0	1	0	77
21-26e	84.679	29.471	718.1	0	0	1	36
22-27m	94.421	27.03	2487.3	1	0	0	47
22-27d	88.534	25.138	1192	0	1	0	77

Where, m-Time 09:00-10:00, d-12:00-01:00 and e-03:00-04:00

Date- 6th April to 27th April, 2014

Table 4: Initial data of insect pollinators

date	Api- cera	Api- mell	Ad- resp	La- mp- boe	Ve- spa	Pi- er- bra	Co- li- era	Co- li- fie	He- li- ar- m	Pr- ec- al- m	Pol- is- sp	Pi- er- can	Po- ly- ne- p	Ny- ct- pla	Sy- nt- sp- p	Po- nt- da- p	Ag- la- cas	M- us- do- m	Er- is- ce- r	Ep- is- bal	Ich- ne- umo	M- us- sor- b	A- nt
1-6m	27	5	13	13		3	1		3		1		1			1	1	2	1	1	2	1	
1-6d	63	2	1	5	1	1	1	2	1	3	2	2	2	1		1	1	3	2	1	2	1	1
1-6e	27		2	6		1						1						2				3	4
2-7m	36	3	16	10	3	3	3	2	3	2	1	1		1	1			2		2	2	3	2
2-7d	57	1	4	6	1	1	1	2	1		3	2	3					2		1	2		1
2-7e	30			4									1					2		2		1	
3-8m	23	3	7	4		1	1		3	1		2				1					4	1	1
3-8d	31	1	1	2	2		1	2	1	1	2	1	3			1	1	1		1	2	1	
3-8e	33			1														2				3	1
4-9m	23	1	12	4			1							1	1			1	2	1		1	2
4-9d	32		4	10				3	2			3	3	1				2	2	1		3	1
4-9e	20			5							1			1				2				3	2
5-10m	25	1	4	12			1		3	1		1				1		1			2	1	1
5-10d	58	1	6	12		1		2			1	1	4					4	2			2	2
5-10e	25			2				1	2		1		4	1				1	1			2	2
6-11m	15		1	3		1	1	1			2			2		1		2		1		1	1
6-11d	41	3	1	6		1	2	2	1			2	2	2				2	3			3	2
6-11e	23	1		4					1					1				1				2	2
7-12m	18	3	5	6			1		2			1		1		2		1			1	1	1
7-12d	56	3	3	6		1		3			1	5	4					3	2	3		5	1
7-12e	14			4		2						1								1			1
8-13m	19		1	2		1	2		1		1		1		2		1				1		1
8-13d	18	2	5	3		2		2	1		2		5									1	
8-13e	11			1		1			2										1				1
9-14m	44	1	14	16		1	1	1	1		2			1			3		3		1		1
9-14d	42	4	6	7		2	1	4				1	4	1				3	2				2
9-14e	15	1		1	1														1			2	
10-15m	33	1	6	15			2	1	2		1	1	1	1	1	1	1	1	2	2	2	1	3
10-15d	43	3	2	7				1	1			1	3	1				2		1		1	
10-15e	36			2					3			1							3	1			3

e																						
11 - 16 - m	28	1	2	12			2	1	1	2		1		1	1		2		1	1	1	
11 - 16 d	18		1	5				1		1							2				2	
11 - 16 e	22	3		2					2			1		1				1				
12 - 17 m	30		1	37	1													2	2	2	1	
12 - 17 d	26	10		6	1		1		2		3						1				2	
12 - 17 e	35	7	3	7				1	2			1					1	1			1	
13 - 18 m	45	1	4	11	2			3	2	1		2	2					1	6		4	
13 - 18 d	29		2	7				6	4			1	2						1		1	
13 - 18 e	34		2	11		1			1			2		1							2	
14 - 19 m	31	2	2	14		1		3				1						2		5	1	
14 - 19 d	51		2	3		1	1	5				4		1							5	
14 - 19 e	35	3		8		2		1				1	3								3	
15 - 20 m	15	2	3	9		2		#				3	5					2	2			
15 - 20 d	37	8	3	7		1		7			2	1	6	1				1	2		1	
15 - 20 e	20	3	1	5		2		1				5						2	1			
16 - 21 m	17	2	9	14		1		3				3	1	1				1	3		2	
16 - 21 d	25	2		5			1					4						2				
16 - 21 e	25	2		5			1					4						2				
17 - 22 m	31	5	4	3		1	1					2		1				2	3		1	
17 - 22 d	57		12	5		1		3				2					1	3	1		1	
17 - 22 e	10		2	6								1					1		1		3	
18 - 23 m	10	1	8	13			1			1		1	2				1	1		1	2	1
18 - 23 d	29		6	1			2					7	1				2	4	1			

18 - 23 e	37		3	4			1			1	2					1				
19 - 24 m	35		5	8		1	1			4	4	1		1		2	1	2		1
19 - 24 d	34	1	3	5			1	1		2	9					2	7			3
19 - 24 e	20		1	14		2					5									
20 - 25 m	30		15	3		2	1	2			4					1	1			1
20 - 25 d	55		9	1		1		4			5					2	4	1		
20 - 25 e	40		2	7		4	1				1							1		
21 - 26 m	16		12	6		1	5	7			4					1		1		1
21 - 26 d	47		4	3			3	2		2	8		1	1		5	1			
21 - 26 e	22		2	2		1		3			3					2	1			
22 - 27 m	20		9	1		1		5			1	5					2			3
22 - 27 d	53		4	2		1	1	4			6					5	1			

Where, m-Time 09:00-10:00, d-12:00-01:00 and e-03:00-04:00

First column indicates study time from 6th April to 27th April, 2014 and

First row indicates insect species.

Table 5: CANOCO 5 Initial analysis

Analysis 'Interactive-forward-selection', step 'Global permutation test'

Method: CCA

Total variation is 0.91747, explanatory variables account for 20.0% (adjusted explained variation is 11.8%)

Summary Table:

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.0761	0.0462	0.0306	0.0141
Explained variation (cumulative)	8.29	13.33	16.66	18.19
Pseudo-canonical correlation	0.7436	0.738	0.7337	0.4998
Explained fitted variation (cumulative)	41.36	66.49	83.1	90.75