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Research Article

INSECT VISITORS OF WHITE CLOVER (*Trifolium repens* L) AND THEIR RELATION WITH ENVIRONMENTAL VARIABLES IN THE PREMISES OF BHAKTAPUR MULTIPLE CAMPUS, NEPAL

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ABSTRACT

White clover plant, *Trifolium repens* L. one of the important forage crop, entirely depends on insects for its pollination. The research aims to study diversity and relative abundance of insect visitors of white clover and their relation with environmental variables in open ground of Bhaktapur Multiple Campus. Quadrate sampling method was used to explore diversity of insect visitors. Observations of the insects were conducted at morning (09:00 AM to 10:00 AM), noon (12:00 PM to 01:00 PM) and afternoon (3 PM to 4 PM) each day during study period. There were 3472 individuals of insect collected that belong to 20 species in three orders: Hymenoptera (69%), Lepidoptera (27%) and Diptera (4%). Number of individuals of visitors were comparatively higher at noon (1426) than in morning (1256) and lower in afternoon (790 individuals). Number of species found in morning and noon were same (20 species) whereas number of species found in evening was less (16 species). *Apis cerana* and *Lampides boeticus* are the most dominant species belonging to orders Hymenoptera and Lepidoptera respectively. The relation of diversity with environmental variables was correlated by using Multivariate analysis test. The test showed significant effect of light intensity, humidity, temperature and the latter one was the most influential factor on the composition of species.

Keywords: Pollinators, Diversity, Environmental variables, Light intensity, Humidity, Temperature.

INTRODUCTION

Most of the flower visitor insects play a key role as pollinators and help in the maintenance of wild flower reproduction, the products of which support a wide range of invertebrates, birds and mammals (Wilson *et al*., 1999). Cross pollination of most of the agricultural and horticultural crops is brought about by physical agents: wind, water, gravity and biological agents: insects (chiefly the bees), birds and mammals (Kendall & Solomon, 1971).

Trifolium repens is one of 300 species of clover belonging to the Fabaceae family, found over the world (Kentish, 2015). It grows wild as a pasture plant in mountainous areas and is common on lawns whereas it is cultivated as forage crops in various countries: China, India and Pakistan (Pratap, 1997). It is mostly used for grazing, pastures hay, ground cover in horticultural situations and has high nutritive values as it supplies a rich source of proteins and minerals for all classes of lifestocks and wildlife (Frame, 2003). It blossoms from spring to early summer. 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). Commonly called White clover is not easily dispersed by wind, so, insect visitors; chiefly pollinators as bees, flies are needed to transfer its pollen (Duke, 1981).

Physical parameters: temperature, humidity, light intensity, solar radiation and wind speed have influence on the adundance of insect visitors (Abrol, 2010; Sarangi & Baral, 2006). These environmental variables are responded differently by different insects in plant. For example, dipterans act as key pollinators in colder climatic conditions (Gonzalez *et al*., 2009; Keams, 1992) whereas, bees; *Apis mellifera* becomes active between 19^0C and 23^0C and its activity decreases beyond this temperature (Nevkryta, 1957).

White clover plant provides large forage source for pollinators like bees, white butterflies, thickheaded

flies like hoverflies, skippers etc. (Hilty, 2003). It is good source of nectar and pollen for *Apis cerena* and *Apis mellifera*. Beside, these plants are used by farmers of Bhaktapur for their cattles as fodder for high milk production. But this wild plant is neither cultivated for domestic purpose nor studied much in Nepal.

MATERIALS AND METHODS

Observation of insect were conducted from April to June when most of the flower bloom at ground of Bhaktapur multiple campus which lies between 85°25'16.96"E longitude and 27°40'08.38"N. The campus was constructed in front of Bhajyapokhari. About 70% of total area was covered with open land where white clover plants spread.

The insects were collected twice (first and third week) in every sampling month by quadrate sampling method in three different periods each day, at morning (9:00AM - 10:00 AM), noon (12:00 PM - 1:00 PM) and afternoon (3 PM - 4 PM) . The ground with white clover plants within compound was divided into 4 sites. Within each site, 4 quadrates of size 10 × 10 sq. ft were marked randomly. Insects were observed. The representatives of the insect visitors were collected for reference specimen to identify at lab. Most insect specimens were killed in the killing bottle containing ethyl acetate except butterfly which was killed by pressing thorax. Killed insects were pinned with entomological pin and transferred into collecting box. Naphthalene ball was used as preservative. The insect specimens were then identified using various taxonomic literatures (Borror *et al*., 1981; Smith, 2011) and experts' knowledge. Climatic factor in the field i.e., air temperature, air humidity, light intensity were recorded with the help of a device Hobo data Logger.

DATA ANALYSIS

Diversity of insect's visitors was analyzed using Shannon diversity index (H') and its evenness by using PAST program 3.6 versions. Multivariate test of species composition was carried out using a unimodel technique because of presence/absence data and the gradient was short 1.5 SD unit. Though, it used Canonical Corresponding 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 & Smilauer, 2012). Sampling plots and recording time were used 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 Hobo Data Logger.

RESULTS

A total of 20 different insect species were identified and unidentified ant (excluded). The insect specimens represent 3 orders, 10 families and 17 genera. The most abundant species were *Apis cerana* and *Lampides boeticus* which were recorded frequently and *Vespa* spp. was the least frequent visitor (Table 1). Order Hymenoptera (69%) was recorded the most followed by Lepidoptera (27%). Dipterans were the least (4%) (Fig.1). The value of Shannon Diversity was 1.675 (Table 1).

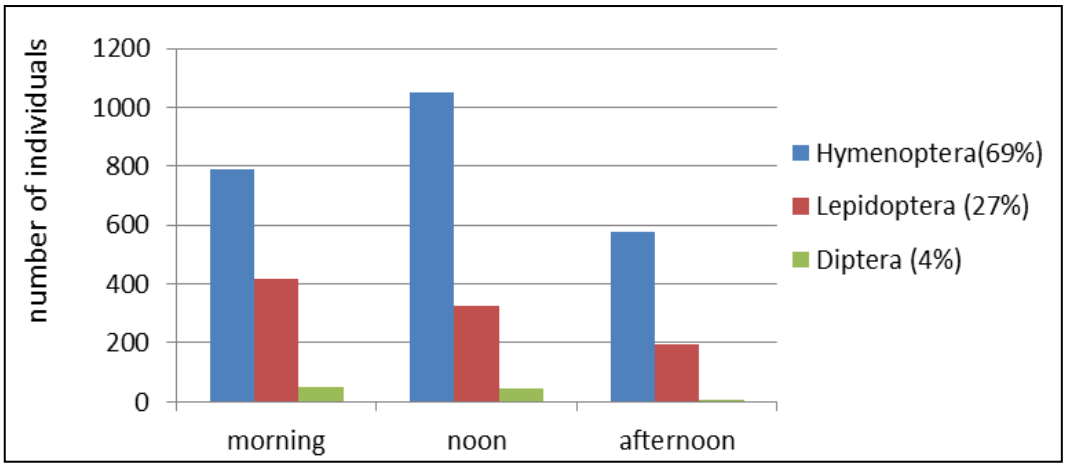

Fig. 1. Number of insect visitors in three different periods of a day.

Table 1: Species and individual number of insect visitors in study site.

Order	Family	Species	MORNING	NOON	AFTERNOON
Hymenoptera	Apidae	*A. cerana* Fabricius, 1793	571	902	534
		A. mellifera Linnaeus, 1758	32	41	20
	Adrenidae	*Andrena* spp. Fabricius, 1775	153	79	18
		Polistes spp. Latreile,1802	8	18	2
	Vespidae	Vespa spp. Linnaeus, 1758	6	5	1
	Ichneumonidae	Ichneumonid spp. Latreile, 1802	20	6	0
Sub- total individual = 2416			**790**	**1051**	**575**
Sub total number of species			**6**	**6**	**5**
Lepidoptera	Pieridae	*Pieris brassicae* Linnaeus,1758	33	86	29
		Pieris candia Sparrman, 1768	20	14	16
		Colias erate Esper, 1805	24	16	2
		Colias fieldi Ménétriés,1855	41	41	20
		Pontidaplidica Linnaeus, 1758	8	3	0
	Lycaenidae	*Lampides boeticus* Linnaeus, 1758	216	114	101
		Polyommatus semiargus Rottemberg, 1775	21	22	9
	Nymphalidae	*Precis almanac* Linnaeus, 1758	8	5	0
		Aglais caschmirensis Kollar, 1848	9	3	1
	Noctuidae	*Helicoverpa armigera Hübner*, 1808	21	14	13
	Arctiidae	*Nyctemera adversata* Schaller,1788	10	8	5
		Syntomoides spp. Hampson1893	7	2	0
Sub- total individual = 942			**418**	**328**	**196**
Sub total number of species			**12**	**12**	**9**
Diptera	Syrphidae	*Eristalis cerealis* Latreille,1804	20	35	12
		Episyrphus balteatus De Geer, 1776	28	12	7
Sub- total individual = 114			**48**	**47**	**19**
Sub total number of species			**2**	**2**	**2**
Total individuals (n) = 3472					
Total species (S) = 20					
eveness (e)			**0.27**		
Shannon index (H')			**1.675**		

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 visitor's 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 insect visitors 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 2).

Table 2: 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 insect species in white clover. The species that were recorded at the highest light intensity were *Syntomis sp.,Aglais cashmirensis*, *Colias erate*, *Precis almona* and *Episyrphus balteatus*. Similarly, high humidity was preferred by *Polistes* sp. and high temperature by *Colias fieldi fieldi*. *Apis mellifera*, *Pieris canidia*, *Lampides boeticus*. And *Apis cerana* were abundance in all time and all condition.

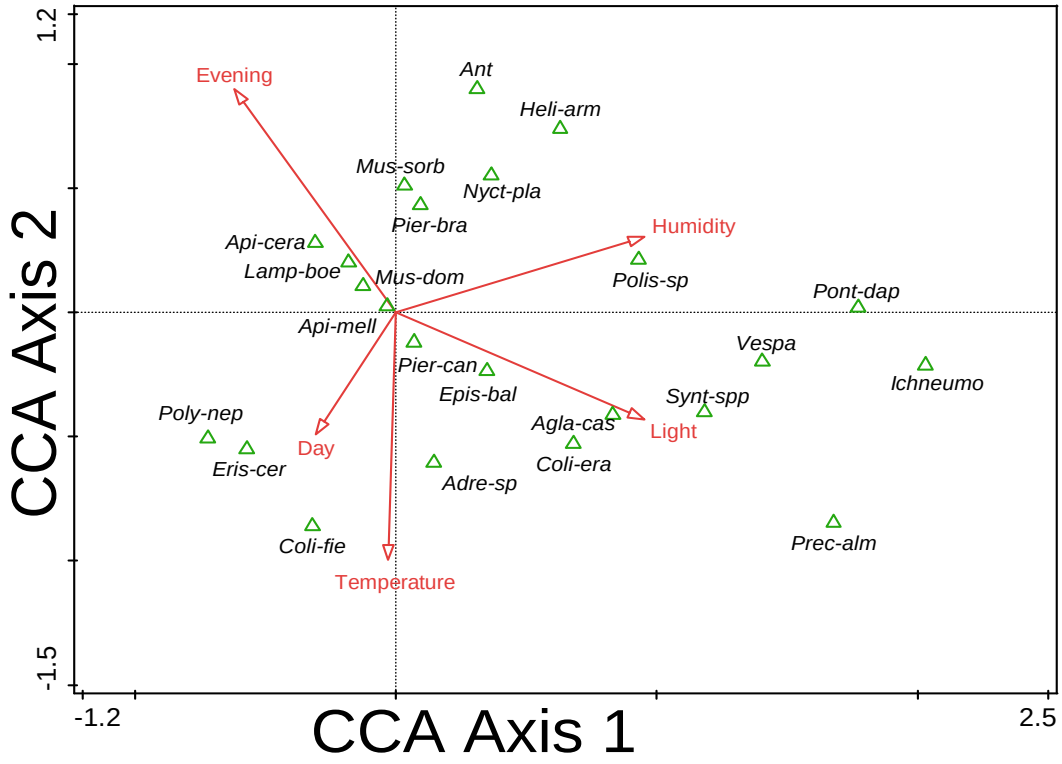

Fig. 2. Relationship between the different environmental factors and distribution of insect visitors.
A. cerana (Api-cera), A. mellifera (Api-mel) Andrena spp (Andre sp.) Polistes spp.(Polis-sp) Vespa spp. Ichneumonid (Ichneumo) spp. Pieris brassicae (Pier bra) Pieris candia (Pier-can) Colias erate (Coli- era) Colias fieldi (Coli-fie) Ponti daplidica (Pont dap) Lampides boeticus (Lamp-boe) Polyommatus semiargus (Poly-sem) Precis almanac (Prec-aim) Aglais caschmirensis (Agla-cas) Helicoverpa armigera (Heli-arm) Nyctemera adversata (Nyct-adv) Syntomoides spp (Synt-spp). Eristalis cerealis (Eris-cer) Episyrphus balteatus (Epis-bal), Musca domesticus (Mus-dom), Musca sorbens (Mus- sorb).

DISCUSSION

Diversity index (1.67) showed diverse visitors in the study field. The number of insects visitors were high in morning however dwindle down with day length except hymenoptera (Table 1). This result was similar with Siregar *et al.* (2016). High diversity and abundance are related with nectar availability as sugar concentration of nectar will be high in the morning (Abrol, 2005) and slowly dilute with day length. The most abundant species observed on *Trifolium repens* was *Apis cerana* (54.75%) belonging to Hymenoptera order and family Apidae. Similar result has been reported elsewhere (Goodwin *et al*., 2011). White clover flowers are strongly adapted to bee pollination; hence bees are mostly attracted for nectar (Kevan and Baker, 1983). In Mt. Carmel, bees belonging to Hymenoptera comprised 90% of the pollinating fauna (Potts *et al*., 2001).

Among butterflies, *Lampides boeticus* are found frequently visiting the clover flowers. It may be due to their habitat preference of this species (Palem *et. al*., 2015) and the study area is dominated by shrub and grassland. Similarly, some moths like *Nyctemera plagifera* and *Syntomis* 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). Though moths are mostly active at night and are attracted by scent and the contrasting white colour (Kevan and Baker, 1983), these moths are active in daytime.

Relation with the environmental factors show, temperature is the most important factor that effect activities of many insects and it depends on the species. In the clover field, *Apis cerana* was found foraging most of the time irrespective of temperature change, however its number was higher at warm temperature. Hence,visitor is influenced by temperature (Bula and Massengale, 1972).

Abundance of insects is also affected by air humidity (Staszeski, 1996). *Polistes* sp. were highly influenced by high humidity. The numbers of butterflies and their activities decrease with increase in moisture. They are active at 50%-60% humidity when nectar secretion is high (Peat *et al*., 2005). In this study, the average temperature and relative humidity were recorded between 1200 and 1300 hours when insect activities were observed high. The study also revealed maximum insect visits between 12:00- 01:00 when the temperature ranges between 28°C-31°C and relative humidity between 20.98%-43% (Table 1 & 2). *A. cerana* are the most abundant when temperature ranges from 28°C-33°C.

CONCLUSION

From the present study, 20 different insect visitors of white clover were identified. Out of them 7 species belong to Hymenoptera, 12 were Lepidopterans and 4 belong to Diptera. *Apis cerana* was the most abundant insect visitors (pollinator) because it is native honey bee species and the study area is located near forest on south direction. The result of the study also shows the importance of honey bee as the pollinator. Butterflies, dipteran flies like houseflies, syrphid flies, etc. are also recorded during the study period. Most of the pollinators ware found to feed nectar and pollen of the clover flower which indicates it as the important foraging vegetation.

The insect visitors are found greatly influenced by the environmental factors like temperature, relative humidity and light intensity. Honey bees are found to be mostly affected by temperature and humidity.

Furthermore, clover plants are found to collect by local farmers for their domesticated cattle as fodder. According to them, it increases milk production.

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