Floral Preference of Butterflies in National Botanical Garden, Godawari, Nepal

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Submitted to

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu

Nepal

May 2023

DECLARATION

I hereby declare that the work presented in this thesis **"Floral Preference of Butterflies in National Botanical Garden, Godawari, Nepal"** has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by the reference to the author(s) or institution(s).

Date: 04 May 2023

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LIST OF ABBREVIATIONS/ACRONYMS

Abbreviated forms	Details of abbreviation
NBG	: National Botanical Garden
BGCI	: Botanical Gardens Conservation International
FCD	: Floral Color Diversity
DPR	: Department of Plant Resources
MoFSC	: Ministry of Forests and Soil Conservation
ANOVA	: Analysis of Variance

ABSTRACT

Butterflies have particular nectar-feeding preferences based on various floral characteristics including flower shape, color, fragrance, nectar composition and flower size. Different species of butterflies prefer distinct varieties of flowers. The survival, reproduction, and pollination of butterflies are impacted by the floral choices they make. The National Botanical Garden (NBG), Lalitpur Nepal holds a large number of a variety of flowering plants and a stronghold of butterfly population offering a set of suitable conditions to test the floral preferences by butterflies. Therefore, this study was conducted to find the floral preference of butterflies based on flower color, flower size, origin of plants (native or alien), type of plants (herbs, shrubs or tress), and the relation between the proboscis length of butterflies and the corolla tube length of flowers in special flower garden of the NBG. The data collection was done from March 2022 to October 2022 where each day 5 hours data were collected in two seasons (spring and autumn) by direct observation method for 32 days. The study blocks (n = 10) of each 5×5 m^2 were designed for the observation. From the study, Pink flower abundance was higher in NBG but the butterflies' visitation was more in yellow flowers (45%) followed by white (15%) and orange (14%). There was a significant difference between the total flower count of different colors and visitation of butterflies in both the seasons ($F=4.762$, $p<0.05$). Also, the butterflies preferred alien flowers over native flower showing a significant difference ($\chi^2= 89.88$, $p<0.05$) in the frequency of butterfly visitation and the type of plant species. Another result suggested that herbs were seemed to be preferred over shrubs indicating the significant difference ($\chi^2=8.28$. $p<0.05$) and small flowers were preferred over the large with the significant difference ($\chi^2=588.66$, $p<0.05$). Similarly, there was a significant positive association between the proboscis length of butterflies and the corolla tube length of flowers ($R^2=0.8619$, $p<0.001$). Findings of this study helps to provide a baseline information and highlight the diversity of butterfly on the basis of their floral preferences which will be much beneficial for their conservation in future. Therefore, additional research efforts should be dedicated to explore the behavioral patterns of butterflies in the future days.

1. INTRODUCTION

1.1 Background

Butterflies are the important pollinating insects under the order Lepidoptera (suborder: Rhopalocera) that serve as a useful indicator of the level of biodiversity in an ecosystem (Parasharya & Jani 2007; Durairaj & Sinha 2015). Lepidoptera is one among the highly specialized insect orders, it may be noted that Antarctica is the only continent on which no Lepidoptera have been found (Shukla & Maini 2015). They can also be named as nature's gardener and are one of the best taxonomically studied group of insects (Shukla & Maini 2015). They are opportunistic feeders visiting a wide variety of flowering plants, thus performing as pollinators in numerous ecosystems (Tiple et al. 2005; Sharma & Sharma 2013). Apart from their ecological significance, butterflies are also renowned as a symbol of peace, happiness, and love, and they hold significant aesthetic and economic worth (Tang et al. 2013).

Butterflies are among the highly developed insects. Their diversity is remarkable, with over 28,000 species worldwide (Ghazanfar et al. 2016). Currently, it has been documented that there are 692 species of butterflies inhabiting Nepal which is a substantial increase from the long term stability of around 660 species (Vander Poel & KC 2022). Distribution patterns of Nepalese butterflies are varied with respect to physiographic zones which include 51% in Terai and Siwalik zone, 88% in middle zone and 13% in the highland zone of the country. About 18% species of the Mid-Hill zones are considered threatened (BPN 1996). The southern part of the Kathmandu valley, extending from Godawari (1360m) to Phulchowki Mountain (2734m) is a species -rich area where more than 150 species of butterflies, mostly forest dwelling species are found (Smith 1989).

A large number of butterfly species are strictly seasonal and have a preference for specific types of habitats. The presence and behavior of butterflies can be used as a measure of ecosystem health, particularly in regards to human-caused disturbances and habitat quality (Kunte 1997). Butterflies can be both generalist and specialists. While some butterfly species are generalists that can thrive in a broad range of habitats. Butterfly species have varying and specific requirements for their habitat, temperature, humidity, larval food plants, and adult food sources, which contribute to their high level of specialization. Each species has its own individual needs in these areas (Hoskins 2012).

Butterflies may select flowers based on their color, shape, size, and/or scent, as they use both visual and olfactory signals. The primary food source for most butterflies is nectar which is the sugary fluid produced from flowers. They tend to have a preference for the nectar of specific plants (Inouye 1980). Flower visitors use their many sensory modalities to distinguish between distinct flower species with colors, morphologies or fragrances (Gumbert & Kunze 2001). The color of flowers is an essential functional trait that has a significant impact on the interactions between plants and pollinators, which in turn contributes to the biodiversity and structure of plant communities (Ômura & Honda 2005). Flower colors serve as sensory cues to pollinators, "advertising" the value and abundance of flower rewards (Aragón & Ackerman 2004). Studies have shown that the visitation of butterflies is influenced by specific floral characteristics, which are considered to be important for their attraction. Flower color could be a good indicator for the various types of pollinators (Fenster et al. 2004). The variation in floral color diversity can be indicative of the interactions and evolutionary history of the signaling mechanisms in shape and color between plants and pollinators (McEwen & Vamosi 2010).

The interactions between plants and pollinators are a vital component of terrestrial ecosystems, and are characterized by a great diversity of interactions. Insect pollinators exhibit various types of flower-visiting behaviors, which have an impact on the dispersal of pollen. Among the foraging behaviors displayed by butterflies are flower selection, the duration of their nectar-gathering activity, and the number of flowers visited during a single foraging session (Pohl et al. 2011). Flower visitation is also influenced by inflorescence morphology, corolla size and depth, and butterfly traits (Tiple et al. 2009). The number of nectar plants present in a particular habitat is strongly influenced by the abundance of herbaceous plant species in that habitat, which is important component for sustaining the abundance of adult butterfly species (Kitahara et al. 2008). Preserving herbaceous plants, particularly those that are native to an area, in habitats with potential for butterfly populations may be a means of enhancing the variety and abundance of butterfly species. Such plants are known to provide attractive floral resources that are beneficial to butterflies (Subedi et al. 2021).

In biology, co-evolution refers to the mutual influence of the evolution in different ways. Different butterfly species prefer different kinds of flowers (Hardy et al. 2007). Numerous interactions between insects and flora that are pollinated by insects are the examples of co-evolution (Ehrlich & Raven 1964). The proboscis is a highly adaptable and essential organs present in pollinators. The feeding preferences of butterflies are influenced by the lengths of both the corolla tube on a flower and the proboscis on the butterfly (Antonini et al. 2006). Butterflies have long and coiled proboscis that allows them to take in liquid food (Krenn & Mühlberger 2002). Species with the long proboscis prefer long corolla flower while species with short proboscis prefer short corolla flowers (Arbulo et al. 2011).

1.2 Research objectives

The main objective of this study was to explore floral preferences of butterflies in National Botanical Garden, Godawari, Lalitpur, Nepal.

Specific objectives:

i. To determine the association of flower visitation frequency of butterflies with flower color and plant types (native or alien, small or large, herbs or shrubs).
ii. To investigate association between proboscis length of butterflies and corolla tube length of flowers.

1.3 Research hypothesis

The study was conducted on the base hypothesis that the butterflies prefer brightly colored flowers than dull colored. They exhibit a preference for certain flower characteristics, such as color, shape, or nectar content, based on factors such as their visual perception, foraging efficiency, or attraction to specific floral scents. Also, they show positive relationship between their proboscis length and corolla depth as they strongly participate in co-evolution with each other.

1.4 Significance of the study

Earlier, Nepali et al. (2018) studied the diversity of butterflies and their relationship with plants at NBG, Godawari. However, the floral preference of butterfly diversity was not carried till date in this area. The NBG provides an ideal condition for establishing the relationship between the visits of butterflies and flower characteristics because the garden has wide varieties of plants; native and alien, tree, shrubs or herbs, flower colors and size, etc. My research was conducted in this area with the aim of filling gaps in knowledge about different types of plant species. i.e., their relationship with the floral preference between butterfly species and between proboscis lengths of butterfly with corolla length of flower. As this kind of study has not been performed since many years, it enables us to investigate the association of butterfly on the basis of their foraging behavior. This study helps to provide baseline information and highlight butterfly diversity on the basis of their floral preferences which will be much beneficial for their conservation in future. This study can also add up firm groundwork for additional research which can help for the benefits of butterflies to flowers and vice versa.

1.5 Limitations of the study

This study is only based upon the behavioral pattern of butterflies with different flower categories not dealing with the diversity of butterflies present in the garden. Butterfly behavior and floral availability can vary throughout different seasons. Therefore, this study was conducted within a limited time frame due to which the full range of seasonal preferences and patterns could not captured.

2. LITERATURE REVIEW

Many biologists have done great contribution in the field of butterflies' diversity to conserve threatened species in Nepal. Various studies on butterflies from many regions of Nepal have been conducted but the study were more focused on diversity, distribution and taxonomy of butterflies (Bhusal & Khanal 2008; Thapa & Bhusal 2009; Khanal 2013). Only a limited number of studies have been conducted on the interactions between butterflies and plants (Nepali et al. 2018; Shrestha et al. 2020; Subedi et al. 2021).

2.1 Preference of flower color and plants by butterflies

Flower color plays a significant role in helping pollinators locate flowers, making it an essential characteristic among floral traits (Reverté et al. 2016). A variety of flower-visiting behaviors can be shown by insect pollinators which influence in the dispersal of pollens. The foraging patterns of butterflies can be characterized by a range of behaviors related to flower selection, time spent at a nectar source, and the number of flowers visited during a foraging session (Pohl et al. 2011). Yellow, orange, pink, red, white and blue are visited by butterflies. It is an ecologically significant behavior exhibited by many butterfly species during pollination (Tudor et al. 2004). Several research have looked into butterfly-plant interactions (Nimbalkar et al. 2011; Arya et al. 2020; Shrestha et al. 2020; Subedi et al. 2021). The study on floral preferences of butterfly species in the Rupa Wetland of Nepal done by (Subedi et al. 2021) documented that the total number of butterfly visits was considerably impacted by diverse plant categories, including a preference for herbaceous plants over woody ones, as well as a preference for flowers with yellow, white, and purple colors over pink ones.

A study on flower color preferences of *Aporia bieti* (Lepidoptera: Pieridae) studied by (Zhang et al. 2018) in the Xiama Forest Farm, Gansu, China explored that yellow flowers were preferred more over red, pink, purple and white flowers which shows that butterflies are related with the floral color choices. Similarly, most of the study shows that butterfly prefers mostly bright color flowers i.e. yellow over other flower (Blackiston et al. 2011; Santhosh & Basavarajappa 2016). Flowers of different colors were found to attract different levels of visits from butterflies. White and pink flowers received fewer visits compared to other colors, while red, yellow, and blue/purple flowers were visited more frequently (Tiple et al. 2005).Visits by butterflies to flowers of trees were less frequent in comparison to their visits to flowers of herbs and shrubs (Nimbalkar et al. 2011). Similarly, exotic flowers preferred more with extended proboscis where high correlation was found between preference for native or exotic plants and proboscis length (Bergerot et al. 2010).

2.2 Relationship between flower morphology and proboscis length

The foraging behavior of insects is influenced by the chemical and physical properties of plants, and the secondary metabolites present in them are believed to play a key role in determining the quality and quantity of foraging resources (Qin & Wang 2001). The visual and olfactory cues used by insects helps to find and direct others to suitable food for foraging. Butterflies have different foraging preferences that is influenced by the compatibility of flower morphology, specifically the length of the corolla, with their own morphology, particularly the length of their proboscis (Tiple et al. 2009; Bergerot et al. 2010). Butterflies tend to visit flowers whose corolla characteristics match the length of their proboscis, and also take into consideration flower color, shape, and structure, which are considered as important visual signals that attract them (Tang et al. 2013). Therefore, many studies on butterflies can offer valuable insights into the co-evolution of insects and plants. Butterflies tend to have more interactions with flowers for foraging if the length of their proboscis is compatible with the length of the corolla of the flowers they visit. This compatibility in morphology plays a key role in determining their foraging preferences.

The research done in Rupa wetland of Nepal by (Subedi et al. 2021) found a positive correlation between the length of the proboscis of butterflies and the length of the corolla tube of flowers they visit. This suggests that the longer the proboscis of a butterfly, the longer the corolla tube of the flowers they prefer to feed. Zhang et al. (2018) investigated the adaptive relationship between the size of the proboscis of *Aporia bieti* butterflies and the length of the corolla tube of flowers. They found a significant correlation between the two, indicating co-evolution between them. Shorter proboscises of butterfly species are more likely to visit flowers with shallow corolla tubes, which are often yellow or white in color (Johnson & Steiner 1997; Krenn 2010). The length of a pollinator's proboscis is an adaptation to access the nectar of flowers with deep corolla tubes. Deeper corolla tubes require pollinators with longer proboscises to access the nectar. Likewise, multiple research have found the significant relationships between corolla of flower and proboscis length in butterflies (Corbet 2000; Agosta & Janzen 2005; Sultana et al. 2017; Mertens et al. 2021).

3. MATERIALS AND METHODS

3.1 Study area

The National Botanical Garden (NBG) is situated in the central region of Nepal at an altitude of 1515 meters. It was established on October 28, 1962, under the Department of Plant Resources (DPR) of the Ministry of Forests and Soil Conservation (MoFSC) of the Nepalese government. Its geographic coordinates are between 83°55'56" to 83°58'50" E longitude and 28°11'40" to 28°12'25" N latitude (DPR 2021).

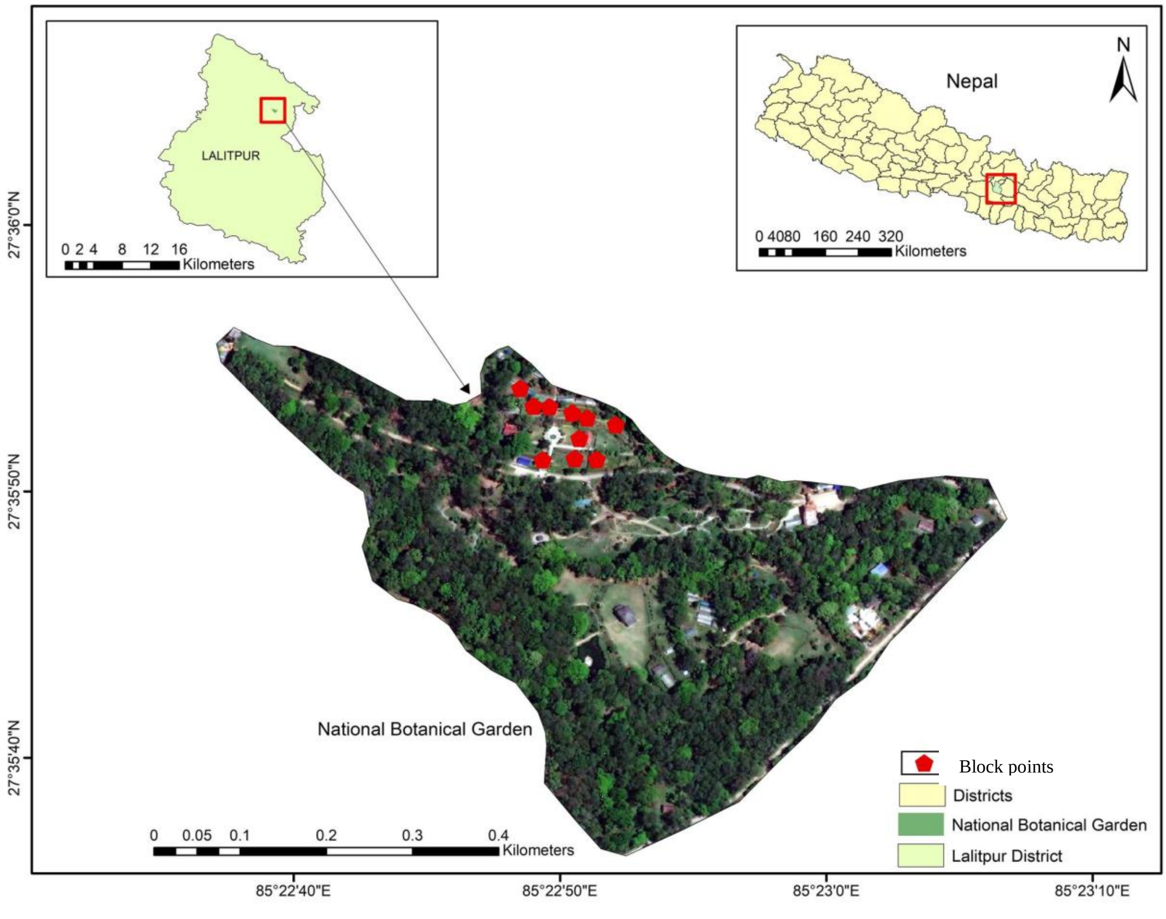

Figure 1. Map of the Lalitpur district showing National Botanical Garden showing the study blocks.

The National Botanical Garden (**Figure 1**) is an internationally renowned botanical garden and a member of Botanical Gardens Conservation International (BGCI) since 2015 AD. This botanical garden's natural landscape is subdivided into 30 thematic gardens (= subgarde ns) located between 1,480 and 2,000 metres. It is located at Lalitpur district, Nepal which is situated at the foothills of Mt. Phulchowki (2765 m), which is the highest peak in the

Kathmandu valley, and is located approximately 10 km southeast of Satdobato in the Lalitpur District of the Bagmati Province. It is further adorned by a natural stream called Godawari that flows through the center of the garden, enhancing its scenic charm (DPR 2021).

The temperature in summer ranges from 20–30º C while in winter it is 0–18ºC and average total rainfall is 18863.5mm. The botanical garden occupies an 82-hectare area with varying topography and exposure, and it is surrounded by evergreen natural forests (Lamichhane & Rai 2021). Over 1000 plant species, including endemic, native, indigenous, naturalized, and exotic angiosperms, gymnosperms, pteridophytes, and bryophytes, are conserved in this botanical garden (Parmar et al. 2022). The total number of 684 species of herbs, 147 shrub species and 201 species of trees are present here. It is popular for the more than 300 species of butterflies and moths (Parmar et al. 2022). The primary objectives are the in-situ and ex-situ conservation of plants, as well as teaching, research, and leisure. Since its establishment, it has been accessible to the general public (Lamichhane & Rai 2021).

3.2 Methods

3.2.1 Preliminary field Survey

The preliminary field visit was done to explore the abundance of butterfly and presence of different colored and types of flower in NBG during the month of March, 2022. Then special flower garden of NBG was selected as the study site of my research as the abundance of flower varieties along with the butterfly population was available there.

3.2.2 Data collection

The data was taken during two seasons of a year 2022 i.e. spring (April and May) and autumn (September and October) where maximum number of butterflies are observed. The data was not collected during the winter season because butterflies are not usually present during this period as they are sensitive to cold temperatures (McDermott Long et al. 2017). The observation was carried out for 32 days i.e., 5 hours a day between 10 a.m. to 4 p.m. in each season. Total no. of ten study blocks of 5×5 m^2 were arranged where maximum butterflies can be observed and the number of frequencies of each species of butterflies visiting particular flower and the types of plant was recorded. Each block was observed for 30 minutes each. Butterflies were explored by direct observation method by observing no. of frequencies of the butterflies visiting different plant species under all categories like color, type of plant species, etc.

To investigate proboscis length and corolla tube length, feeding butterflies were caught in each block using a sweep net. The measurement of the proboscis length was carried out by gripping the unfurled tip of the proboscis with a needle or forceps and measuring the distance from the base to the tip (Ehrlich & Raven 1964). In addition, the flowers on which the observed butterflies were feeding were collected and their corolla tube length was measured. The corolla's depth was determined by measuring from the base to the point at which the butterfly could access the nectar. The data on the plant species, including their category (herb, shrub, or tree), origin (native or alien), flower color, and size (small or large) were also collected.

3.3 Data analysis

For the data analysis one-way ANOVA test was done to find out the significant difference of frequency of butterfly visitation with different flower colors which provides insights into the potential influence of flower color on butterfly behavior and preferences.

Similarly, Chi square test was performed to determine the significant association between the different plant categories and the frequency of visitation of butterflies. This analysis helps to provides insights into the potential influence of flower categories on butterfly visitation behavior.

It was performed by using the formula:

$$\chi^2 = \frac{\sum(\mathbf{O}-\mathbf{E})^2}{\mathbf{E}}$$

Where, χ^2 is the chi square test statistic

O is the observed frequency in cell

E is the expected frequency in the cell

For the analysis of relation between the depth of the corolla in flowers and the length of butterfly proboscis Pearson's correlation coefficient was calculated. The correlation coefficient is calculated to quantify the strength and direction of the linear relationship between two continuous variables. This allowed to examine whether there was a statistically significant relationship between the two variables. This analysis helps determine the extent to which these variables are associated and provides insights into the potential adaptation and co-evolution between butterflies and flowers. It was calculated by using the formula:

$$r = \frac{n(\sum xy) - (\sum x)(\sum y)}{\sqrt{\left[n\sum x^2 - \left(\sum x\right)^2\right]\left[n\sum y^2 - \left(\sum y\right)^2\right]}}$$

All the analyses of butterfly-plant associations were performed using R version 4.0.2.

4. RESULTS

4.1 Floral preference of butterflies

4.1.1 Flower visitation frequency of butterflies based on flower color

The (**Table 1**) shows the relative frequency of different flower colors in the study area. Pink flowers were the most abundant i.e. 30.66% followed by yellow (22.85%). Other colors, such as red, white, orange, purple, and blue, had varying levels of relative frequency ranging from 2.29% to 20.97%. The data provides an overview of the distribution of flower colors in the study area.

Table 1. Table showing relative frequency of flower present in the study area

Flower Color	Flower Count	Relative Frequency (%)
Yellow	4086	22.85
Red	411	2.29
White	2748	15.37
Orange	726	4.06
Purple	3750	20.97
Blue	675	3.77
Pink	5482	30.66

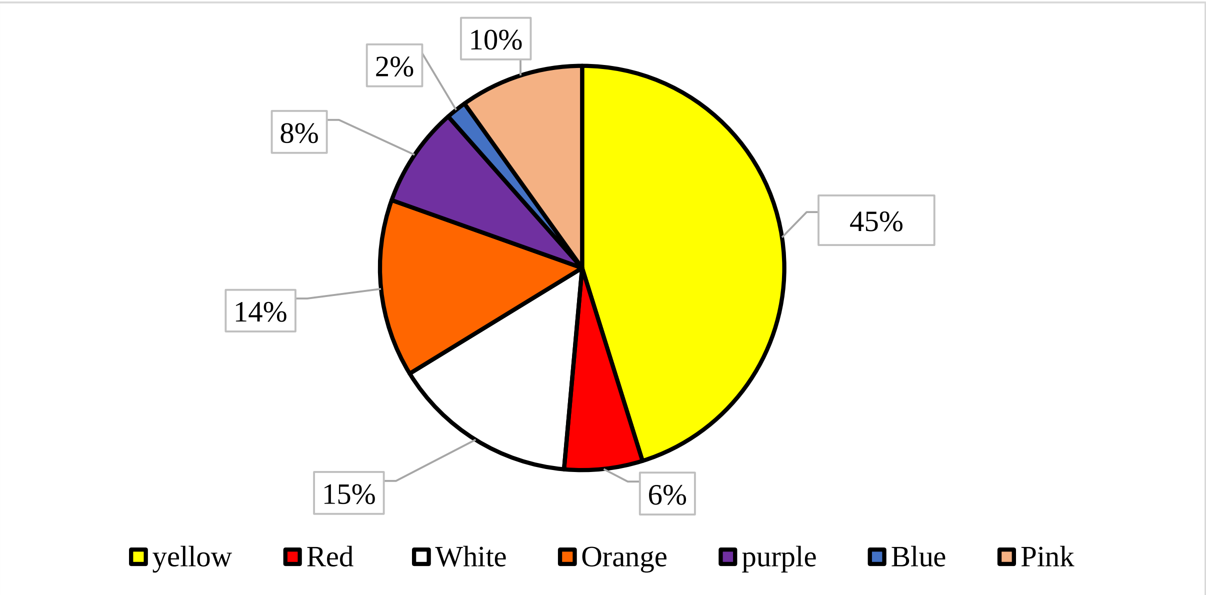

Figure 2. Pie chart showing floral preference of butterflies on the basis of colors

In the study, the frequency of butterfly visitation to different flower colors was assessed. The data (**Figure 2**) suggests that yellow had relatively higher visitation rates compared to other colors, with yellow (45%) followed by white (15%) and orange and (14%). Blue flowers had the lowest visitation rate at 2%. These results indicate potential preferences among butterflies for certain flower colors in terms of visitation frequency. ANOVA test revealed the difference was statistically significant ($F=4.762$, $df=1,12$, $p<0.05$) between the total flower count of different colors and visitation of butterflies in both the seasons.

4.1.2 Seasonal preference of butterflies on different flowers

The data presented (**Figure 3**) shows the frequencies of butterfly visitation to different flower colors during the spring and autumn seasons. Yellow flowers had the highest visitation frequency in both seasons, while blue flowers had the lowest. Red, purple, and white flowers received moderate visitation rates, while orange and pink flowers had varying frequencies. These findings highlight the preference of butterflies for certain flower colors, with yellow being the most attractive. The ANOVA test revealed that there was no significant difference in the visitation of butterflies in different colored flowers in two seasons ($F=2.444$, $df=1,12$, $p>0.05$)

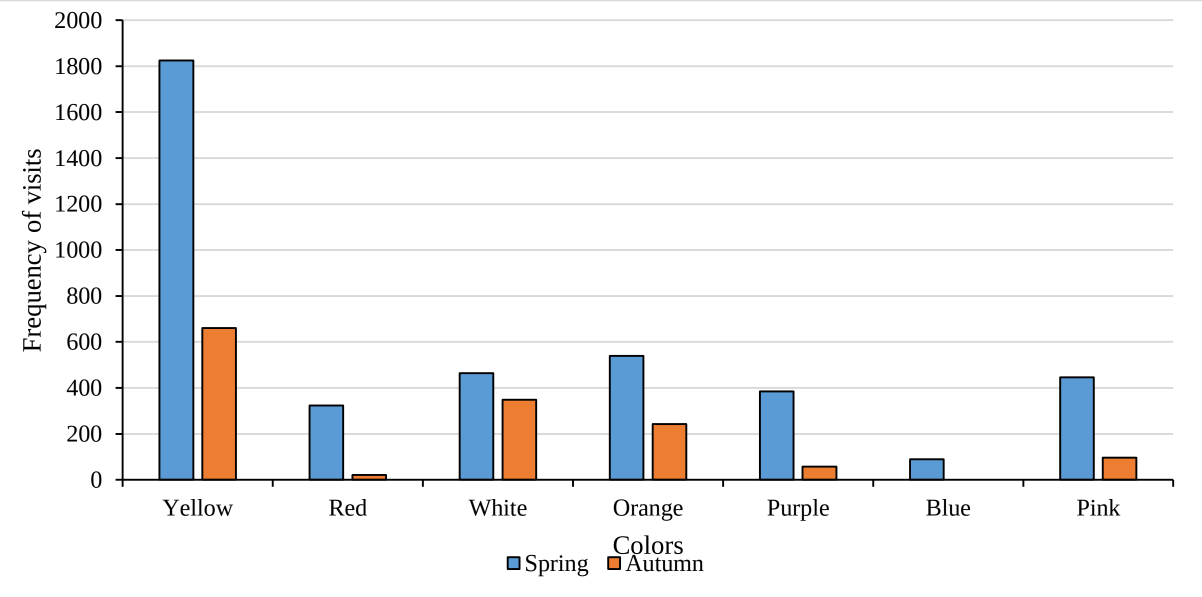

Figure 3. Seasonal variation in flower visitation frequency based on flower color

4.1.3 Floral preference of butterflies on the basis of origin of flower

In spring, alien plants attracted more butterfly visits (3530) compared to native plants (542) (**Table 2**). Similarly, in autumn, alien plants (1071) are still more visits than native species with visitation (341). The variability in visitation frequency was higher for alien plants in both seasons. The Chi-square test revealed a significant difference in the frequency of butterfly visitation and the type of the plant species i.e. native or alien in both spring and autumn seasons (χ^2=89.88, $p<0.05$).

Table 2. Table showing the mean frequency of visitation with Standard deviation for both native and alien species in summer and spring seasons

Season	Plant Species	Frequency of Visitation	Daily Count Mean	Standard Deviation
Spring	Native	542	16.9375	2.63155
	Alien	3530	110.3125	12.72724
Autumn	Native	341	10.65625	2.02462
	Alien	1071	33.46875	6.01318

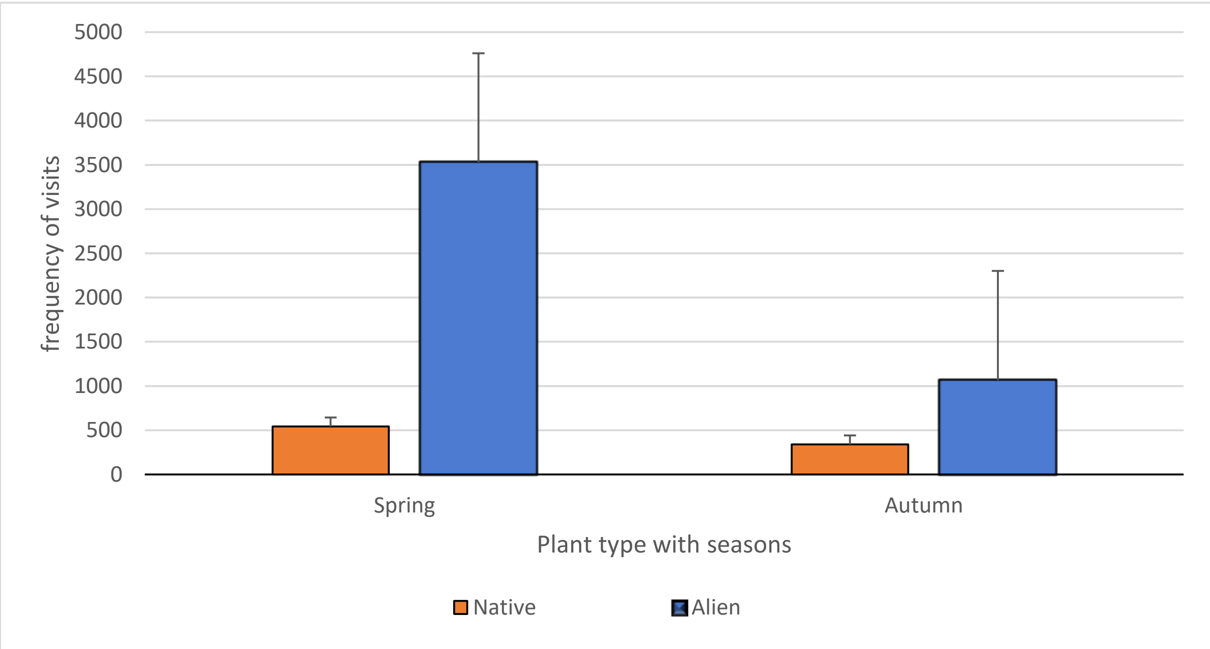

Figure 4. Bar graph showing the daily count mean of visits on the basis of season and origin of flower

4.1.4 Floral preference of butterflies on the basis of size of plant

The data reveals that during spring, both herbs and shrubs have higher average daily counts compared to autumn (**Table 3**). Herbs have an average count of 98.66 in spring, which drops to 34.13 in autumn. Similarly, shrubs have an average count of 31.25 in spring, which decreases to 8.63 in autumn. In summary, spring exhibits higher plant abundance with more variation in counts, while autumn has lower counts with less variation. The Chi-square test revealed a significant difference in the frequency of butterfly visitation and the type of the plant species i.e. herbs, shrubs or trees in both spring and autumn seasons (χ^2=8.28, $p<0.05$). This means that the observed differences in the frequency of butterfly visits between the plant categories are not likely to be due to chance.

Table 3. Table showing the daily mean count with standard deviation in three plant categories i.e. herbs, shrubs and trees in both seasons

Season	Plant types	Daily mean Count	Standard Deviation
Spring	Herbs	98.66	35.57
	Shrubs	31.25	11.18
Autumn	Herbs	34.13	11.54
	Shrubs	8.63	2.91

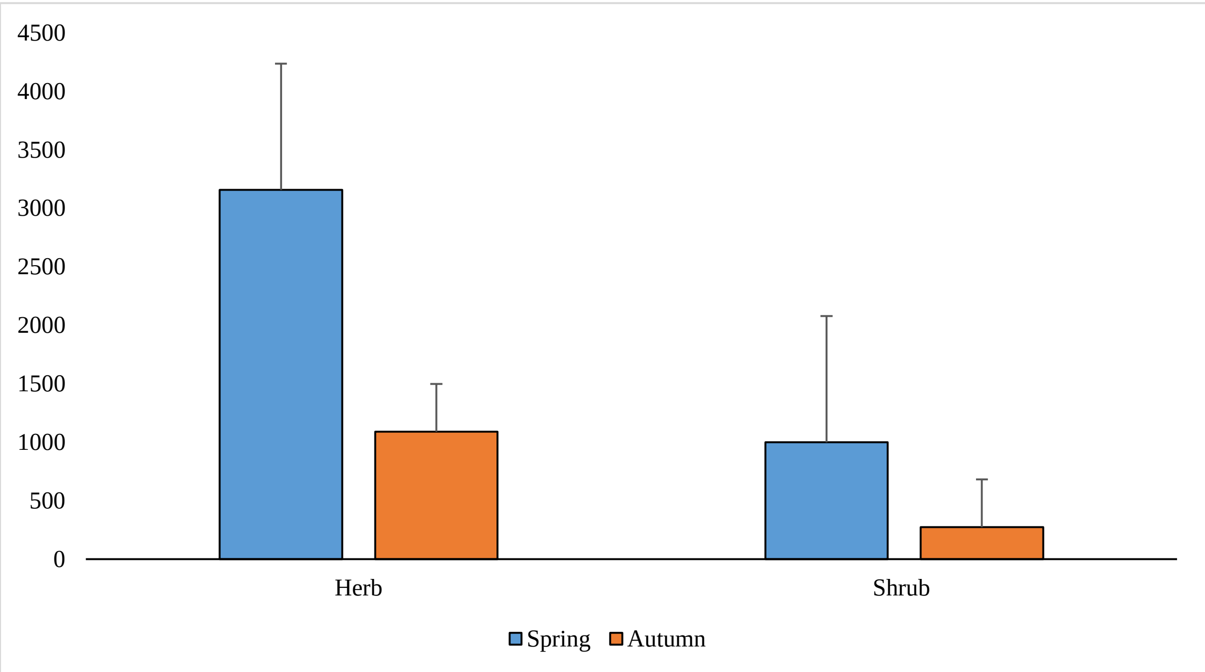

Figure 5. Bar graph showing the daily count mean of visits on the basis of season and type of plant

4.1.5 Floral preference of butterflies on the basis of size of flower

The median value was calculated where the flowers with diameter less than 5 cm were placed as small flower and more than 5 cm as large flowers. The observed data shows the visitation of butterflies for small flower and large flower plant species during the spring and autumn seasons (**Table 4**). In spring, the small flower species receives a higher frequency of visitation with a count of 2543, indicating that it attracts more visitors during this season. The large flower species, although slightly less visited, still receives a considerable frequency of visitation with a count of 1515.

In autumn, both the small flower and large flower species experience a decrease in visitation frequency. The small flower species receives a lower count of 357, indicating a decrease in visitor interest during this season. The large flower species, on the other hand, still maintains a relatively higher frequency of visitation compared to small flowers in autumn, with a count of 1055. From the chi square test, a significant association between the frequency of visitation of butterflies and the type of flowers (small vs large) with (χ^2=588.66, $p<0.05$) was obtained.

Table 4. Table showing the daily count mean with standard deviation for small and large flower in both seasons

Season	Plant Species	Frequency of Visitation	Daily Mean Count	Standard Deviation
Spring	Small flower	2543	79.47	37.17
	Large flower	1515	47.34	28.15
Autumn	Small flower	357	11.16	9.08
	Large flower	1055	32.97	27.91

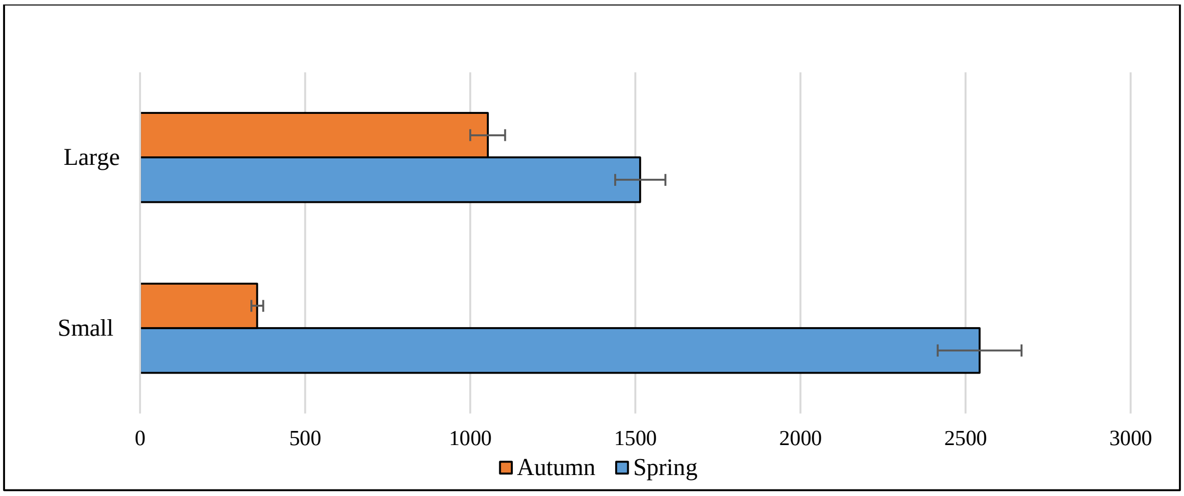

Figure 6. Bar diagram showing the daily count mean of visits on the basis of season and flower type

4.2 Association between proboscis length and corolla tube length of flowers.

The Pearson correlation analysis for the proboscis and corolla length was found to be strongly positively correlated ($R^2 = 0.8619$, $P<0.001$) which suggests that approximately 86.19% of the variation in proboscis length can be explained by the variation in corolla length. This high R^2 value indicates a strong linear relationship between the two variables. The proboscis length of the butterflies were significantly associated with the corolla length of the flowers suggesting the close association with each other.

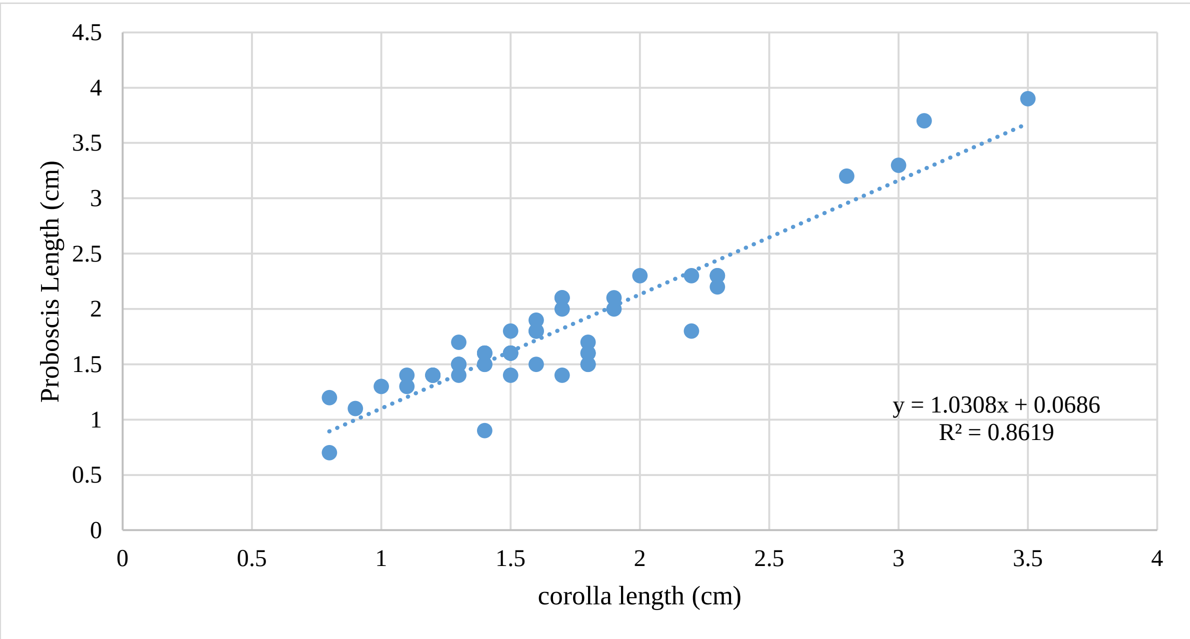

Figure 7. A scatter plot showing significant positive correlation between proboscis length of butterfly and corolla length of flowers.

5. DISCUSSION

5.1 Floral preference of butterflies

5.1.1 Color preference of butterflies

The study demonstrated that various plant characteristics, such as flower color, origin, and size, significantly influenced butterfly visitation. There was a significant difference between butterfly visitation in two different seasons i.e. spring and autumn in different flower colors. Butterflies visited more in yellow color flower followed by white, orange, pink, purple, red and blue flowers respectively. This might be due to the fact that the butterflies prefers more on light colored flowers than dull colored. There has been plenty of variation reported in the findings of previous research on floral color preferences on butterflies. The result was supported by Subedi et al. (2021) where butterflies showed a significant preference for flowers with yellow, white, and purple colors, while they visited pink flowers less frequently.

The findings shows contrast with (Zhang et al. 2018) where the most frequently visited flowers in the field were yellow, followed by red, purple, pink, and white suggesting that color is the most important visual signal that attracts butterflies during foraging. Another study conducted in India discovered that the butterflies preferred yellow color more followed by white, pink and blue colored flowers (Santhosh & Basavarajappa 2016) providing a possibility that yellow flowers might have offered more nectar to visiting pollinators. Similar finding were supported by (Tiple et al. 2005) where the frequency of butterflies visitation was found significantly higher in yellow, red, blue and purple when compared to white and pink flowers.

Sharma & Sharma (2013) studied the utilization of nectar resources where more preference was found to be on white followed by yellow, red and green showing more preference on bright colors. Likewise, Raju et al. (2004) found that butterflies use white, cream, violet, orange, red, yellow, purple colored flower for their forage among which white colored flower were found to be more preferred . Similar findings were obtained in my study on color preferences of butterflies, which is consistent with the research conducted by (Blackiston et al. 2011). Their study showed that monarch butterflies selected yellow flowers less than half as often as orange ones, and blue flowers were chosen less frequently than yellow, with red being the least preferred color.

Different butterfly species gave different amounts of importance to visual cues which directly affects in their foraging choices. (Ômura & Honda 2005) found that show a high preference for yellow, purple, and blue colors. The research conducted by (Briscoe et al. 2003) revealed that there is a correlation between the color preferences of closely related butterfly species when foraging for nectar. The study suggests that this similarity in color preference may be due to the phylogenetic characteristics of their color vision, indicating that the evolution of color vision may have played a role in shaping the foraging behavior of butterflies.

5.1.2 Floral preferences of butterflies in various plant categories

The study revealed that butterflies prefers more on exotic flowers than native flowers. Bergerot et al. (2010) found that butterflies with lengthy proboscis were more common on exotic flower as they are deeper than native flower. Exotic flower are taller and had greater floral diameter than natives. The preference for native and exotic plants is strongly correlated with the length of the proboscis. Furthermore , butterflies are known to be rapid learners who prefer high rewarding colors above natural color preferences (Kandori & Yamaki 2012).

Similarly, high floral preference of butterflies' visitation was found to be more on herbs followed by shrubs and trees in my study. (Tiple et al. 2005) investigated the frequency of butterfly visits to flowers of different plant categories. The results showed that butterfly visits were more frequent to flowers of herbs and shrubs compared to trees. As Butterflies frequently brood and need access to a continuous supply of flowering nectar-producing plants which may not always be present. Flowering time may be more crucial in tropical environments than it is in temperate zones where herbs and shrubs significantly provide this more frequently than trees. (Raju et al. 2004) studied on nectar host plants of some butterfly species where butterflies get floral nutrients more herbs and shrub species in rainy season and whereas trees in dry season.

From my study, I found that the butterfly visitation was found to be more on small flowers than large flowers. Similar study done by (Sharma & Sharma 2013; Zhang et al. 2018) revealed that the butterfly preferred small flowers than big ones. This was explained by a butterfly population being so huge that food resources became scarce causing the population to adjust by acquiring shorter or longer proboscises. In contrast, a study showed that large flowers are more visible than smaller ones, making them simpler to locate when distance and other factors are equal (Spaethe et al. 2001). Insects' compound eyes have limited spatial resolution, making it much simpler and quicker for them to locate larger flowers than smaller ones. Larger blossoms may also result in greater rewards. Typically, nectar volume increases with the size of flowers' inflorescences and corollas, indicating that larger flowers typically offer greater nectar rewards (Blarer et al. 2002).

5.2 Correlation between proboscis length of butterflies and corolla tube length of flowers

From my research findings, it is evident that a correlation exists between the length of butterflies' proboscises and the depth of the corolla in the flowers they visit. Specifically, butterflies with shorter proboscises tend to prefer flowers with shorter corolla tubes, while those with longer proboscises are more likely to visit flowers with deeper corollas. As per data, correlation coefficient value was calculated as 0.8619 which indicated the proboscis length of butterfly and corolla length are more significantly correlated with each other. (Subedi et al. 2021) also found similar result where the proboscis and corolla length were significantly correlated with each other (r= 0.466). According to Corbet (2000), the maximum depth of a flower's corolla limits the species that are able to feed on its nectar to those with proboscises of sufficient length. This refers that butterfly species with shorter proboscises are incapable of accessing nectar from flowers with deep corollas

Ranta & Lundberg (1980) reported that the species with the greatest range of corolla tube depths had the longest proboscises. Thus, a lengthy proboscis enables feeding on the wider range of floral plant species. (Szigeti et al. 2020) investigated that the correlation between the proboscis length of *Clouded Apollo* butterflies and their flower visitation, and it was found that butterflies with longer proboscis tend to feed on flowers with deeper corollas. The proboscis length is an important factor found by (Sultana et al. 2017) in the coevolution between butterflies and the flowers they feed on. It also revealed that butterflies can only feed on flowers whose corolla depth falls within their proboscis length range. Similarly, Tiple et al. (2009) demonstrated that Papilionids fed on the flowers with long corolla tubes in central in India. Mertens et al. (2021) detected a positive relationship between the lengths of proboscis of hesperoid butterflies and tube of visited flowers in tropical rainforests of Mount Cameroon.

6. CONCLUSION AND RECOMMENDATION

6.1 Conclusions

The National Botanical Garden was ideal place to investigate the floral preferences of butterflies because of the availability of various plant characteristics such as flower color, origin, and size. The results revealed that multiple factors influenced butterfly foraging behavior, including the category of the plant, the color of the flowers, the type of corolla, and the length of the corolla tube. Regarding flower color, butterflies showed a preference for yellow, white, and orange flowers, while exhibiting lesser preference for pink, red, and blue ones. Interestingly, this preference did not significantly vary across different seasons, indicating a consistent color preference throughout the year. In terms of plant origin, the study discovered that exotic plants were more frequently visited by butterflies compared to native plants. Furthermore, butterflies exhibited a preference for herbs and shrubs over trees, as well as for smaller flowers over larger ones. A notable finding was the observed statistical correlation between the length of the butterflies' proboscises and the length of the corolla tubes in the flowers they visited. This suggests that the proboscis length of butterflies influences their ability to access nectar resources in relation to the length of the floral tubes. The study highlighted NBG as a suitable habitat for various butterfly species due to the availability of a wide range of flower species. By investigating the floral choices of butterflies across different plant categories and establishing the relationship between proboscis length and corolla length, this research contributes to a better understanding of butterfly ecology and their interaction with floral resources in the National Botanical Garden.

6.2 Recommendations

Some recommendations which can be suggested are mentioned below:

- More research works related to the foraging behavior must be prioritized more as its study is rare in Nepal.
- As NBG is crowded with more human disturbance, the habitat must be managed in such a way that they are suitable for maintenance of butterfly diversity.

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APPENDICES

Appendix 1. Data sheet for collection of floral visitation into different categories

Block no: …………………. **Date:…………………….**

S.N	**Frequency of visit of butterflies to different flower colors**							**Flower origin**		**Type of plant**			**Flower type**	
1.	**Y**	**R**	**W**	**O**	**P**	**B**	**Pi**	**Native**	**Alien**	**Herbs**	**Shrubs**	**Trees**	**Large**	**Small**
2.														
3.														
4.														
5.														
6.														
7.														
8.														
9.														
10.														
11.														
12.														

Time: ………...................

Appendix 2. Data sheet for collection of proboscis length of butterflies and corolla length of flower

S.N	Proboscis length of butterfly	Corolla length of flower
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		

PHOTO GALLERY

Junonia atlites

Junonia iphita

Athyma jina

Junonia almanac

Argyreus hyperbius

Symbrenthia lilaea

Photoplate 1. Butterflies species visiting different colored flowers

Dodona eugenes

Eurema hecabe

Junonia iphita

Aglais caschmirensis

Argyreus hyperbius

Cethosia biblis

Photoplate 2. Butterflies species visiting different colored flowers

Photoplate 3. Capturing butterflies using sweep net

Photo plate 4. Unfurling the coiled proboscis using pin

Photo plate 5. Measuring the proboscis length