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Research Article

Diversity of pollinators in marigold (*Tagetes erecta* L.) in a core area of Kathmandu Valley, Nepal

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Abstract

Insect pollinators provide crucial ecosystem services in the urban environment but are under threat and lack comprehensive study and Pollinators' interaction to change urban land use. Marigold is an important nectar provider for many insects, plays a major role in ecosystem services, and holds a strong cultural and economic value. Therefore, it is important to learn the diversity of insect pollinators in marigolds in an urban environment and assess the impact of urbanization on pollinators. This study aimed to study pollinator diversity, visit frequency, and shift-wise distribution in the core urban area of Kathmandu valley. A total of 21 species of pollinators were recorded from fourteen families and four orders: Hymenoptera, Diptera, Lepidoptera and Coleoptera. Lepidoptera accounted for the highest diversity index of 1.87 followed by Diptera. Furthermore, the study recorded the highest species diversity and abundance during the daytime compared to the morning and the evening hour. The highest diversity of Diptera and Lepidoptera suggests that the urban area is a suitable foraging area whereas, the absence of wild bees further suggests the urbanization as a threat to the wild bee population and demand urgent policy or action for the conservation of pollinators for the conservation of biodiversity and growth in agricultural production.

Keywords: Diptera, diversity, Hymenoptera, Lepidoptera, marigold

Introduction

The ecological interaction of plants and pollinators in terrestrial ecosystems suggests the shift of evolutionary diversification in both plants and pollinators (Ollerton, 2017). Pollination is widely recognized as an essential ecosystem service and a key process to sustain food security (Klein et al., 2007). Pollination is crucial for food production and human livelihoods. It is therefore very important to understand how pollinators respond to the flower and the environment. Many studies suggest that pollination is important for agricultural production and other flowering plants by establishing food development (Pardo & Borges, 2020). Pollinators are the biotic agent, animal or vector that help to move the pollen from anther to the stigma of another or the same flower to achieve fertilization of a flower (Das et al., 2018). Pollinators enhanced the genetic diversity of around 80% of plant species. They provide various benefits in food quality and quantity, seed production, and fertility, leading to a greater next generation of plant species (Miller et al., 2018; Lee et al., 2015; Gill et al., 2016).

Insect pollinators are the major group of pollinators. Globally, 80,000 species of insect pollinators are estimated to present (Kumar & Naidu, 2010), where the pollination of 87.5% of phanerogams are by animals like birds, bats, lizards, and small mammals (Ollerton et al., 2011). More than 75% of 115 leading crop species are dependent or benefitted from animal pollination, while wind and self-pollination are dependent on only 28 crop species (Klein et al., 2007). It is approximated worldwide that 56.5% of global cultivated crops are bees pollinated, making them the most important pollinator for the natural ecosystem (Miller et al., 2018). Besides bees, other insects like beetles, butterflies and wasps play a major role in pollination at a significant rate. It is approximated that 19% of global crops are flies pollinated, and likewise, 5% are pollinated by wasps, 5% are beetles pollinated, and 4% are butterflies and moths pollinated. Moreover, in the tropical forest, 90% of the plant species are dependent on animal pollination like birds, and bats (Abrahamczyk et al., 2011). In two Amazonian rainforests, 54% of the pants species are pollinated by bees, followed by birds and bats (van Dulmen, 2001). The majority of plant species are visited by various pollinator groups within the community, but the visitation of the species does not determine the effective pollination (Barrios et al., 2016).

Urbanization and habitat fragmentation have various effects on pollinators (Potts et al., 2016). Pollinators' diversity is well studied in the natural habitat and agricultural area, but the suitability of pollinators in urban areas is under-studied and unclear (Baldock et al., 2015).

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Many studies suggest the decrease in species richness of insect pollinators with increased urbanization (Jones & Leather, 2012; Martins et al., 2017; Vanbergen & Initiative., 2013). Core urban areas that include industrial sectors, roads, parking lots displaces the nesting and floral resources that is needed for the survival of bees and other pollinating species (Martins et al., 2017). The increased urbanization and intensification of agriculture have fragmented and destroyed natural habitats that the pollinators are dependent on for the foraging and nesting habitats. The intensive farms often use pesticides that harm the pollinators (Garibaldi et al., 2011; Vanbergen & Initiative, 2018). Moreover, the shifting of the climatic range has affected the pollinators' population, ultimately affecting the population distribution. However, the effect of land use urbanization on pollinators has remained controversial; for instance, some studies show the species richness of pollinators to be positive whereas, some studies show the negative effect (Hou et al., 2019).

Marigold is a widely grown flower and the most critical flower in the context of Nepal. Marigold is a perennial or annual herbaceous flower in the Sunflower family, Asteraceae (Ekka et al., 2018). Marigolds are grown in various lands and demand a mild climate for abundant growth and riotous flowering (Khanal, 2014). In Nepal, marigold has a significant cultural and religious value, especially in the Tihar festival. Marigold is considered a sacred flower used in worshipping gods, decorating houses and used as garlands during various occasions. Because of this reason, this plant holds great economic benefits. Besides its cultural and commercial significance, this flower plays a very vital role in ecological function. *Tagetes erecta* act as a pioneer on poor soils and hence might be suitable for the remediation of areas degraded by metal pollution while providing environment-enhancing green spaces (Coelho et al., 2017). In addition, the flower mainly planted for the Tihar festival that lies in autumn in Nepal and acts as a vital nectar provider for pollinators in the absence of other flowering flowers. But with the rapid urbanization, insect pollinators are in threat that is ultimately threat to the ecosystem function (Dong & Karmacharya, 2018). So far, there have been many studies on the diversity of pollinators on various fruits, vegetables, and oilseeds, but no systematic studies on ornamental crops such as marigold (Shilpa et al., 2014). Therefore, the study aims to study the diversity of pollinators on this flower in the urban areas of the Kathmandu Valley.

Materials and Methods

Study area

The study was carried in Ratna Park, Kathmandu Valley, which lies in Nepal's central part. Kathmandu Valley is one of the most populated urban regions of Nepal. According to the Ministry of Urban Development (MoUD, 2015), Kathmandu valley accounts for 24% of the total urban population; meanwhile, Kathmandu alone contributes 9.7 % of the total population. Ratna Park is an urban park located in the center of Kathmandu Metropolitan City (Fig. 1) and attracts many local and foreign tourists. It was initially opened in 1964 covers a total land of 2.2 hectare. The park lies at 27.706055⁰ N,85.34892⁰E and 1260 m asl elevation. The park is surrounded by many vehicles' focal point, resulting in a large amount of particulate matter (PM).

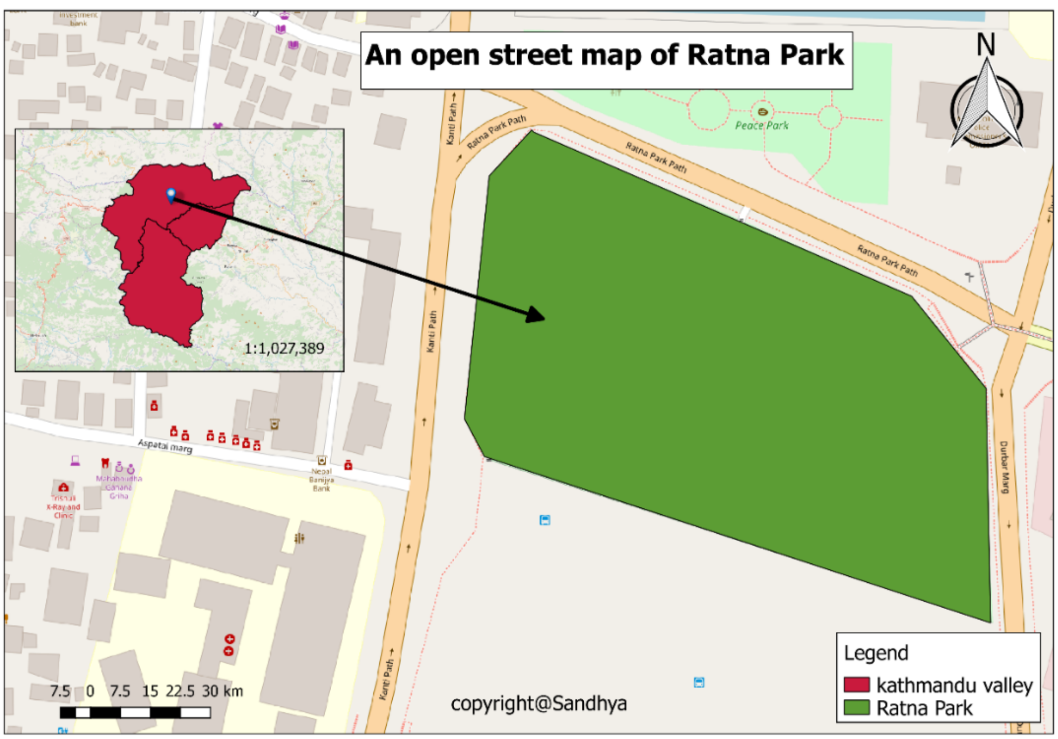

Figure 1 An open street map of Ratna Park, Kathmandu, Nepal

Data collection and analysis

Insect pollinators in marigold were observed in October - November 2020 thoroughly throughout the Ratna Park. The observed flower patches consisted of a total of 20-30 plants of marigold. Some patches of the study site had other flowers beside marigolds such as *Tagetes minuta*, *Bellis perennis* and *Rosa* sp. The flower patches were observed twice a week for two months and three times a day; morning, afternoon, and evening. The length and width of the patches were measured and noted down. It was 20 m in length and 9 m in width. Sample patches were observed for 30 minutes in each shift for two months, which is equal to total time spend in observation is 9450 minutes, and 3150 minutes in each shift. The morning data was collected from 8-8:30 AM, and the other data were collected from 1-1:30PM. Likewise, the data for the evening was collected from 4-4:30 PM. The patches were observed 5m or more apart from each other to avoid the repetition of pollinators in both sample sites. The visitation of the pollinating species in flowers per minute was observed and noted. In addition, the time duration taken by the species in a flower was also noted. Every insect entering the plot was counted as a new individual. Direct observation of the pollinators was done for identification. The species were photographed from various angles to obtain good photographs for valid determination. The elevation and the coordinates of the park and the patches were recorded using the Global Positioning System (GPS).

The species were identified and sorted out by tallying its photograph with various booklets, guidelines, and literature papers. The butterflies were identified by using the booklet provided by ICIMOD (ICIMOD, 2014). Similarly, honeybees were identified by tallying its photographs with the guideline, given by Panthi (2013). Similarly, honeybees were identified by tallying their photographs with the guideline given by Unidentified pollinator species were identified with the help of an Entomologist. Likewise, identified species were further tallied with the preserved species of Natural History Museum, Kathmandu, for a valid confirmation. The species were recorded and classified to correspond to their family, order, genus and species. The diversity and frequency of the pollinators might be recorded less was expected since the study was carried in winter. The statistical test such as the Shannon Wiener Index was performed for the species diversity along with the species evenness and species richness. Likewise, the species abundance and relative abundance were also calculated (Tucker et al., 2017).

Results and Discussion

Insect pollinators diversity

A total of 21 species of pollinators from fourteen families and four orders, namely: Hymenoptera (4 species), Diptera (Six species), Coleoptera (one species) and Lepidoptera (eleven species) recorded in the marigold flowers during the study period (Table 1). Urban areas are presumed to support the habitat of many pollinators and provide a range of resources for pollinating species, which includes nesting sites and food resources (Hennig & Ghazoul, 2011). It can be presumed that the Dipteran and Lepidopteran species were positively influenced by urbanization. A similar result was observed by Hennig and Ghazoul (2011) and Baldock et al. (2015), where they found a higher diversity of Diptera in the urban areas, which further supports the observation of our study. Despite various discussions on the positive effect of urbanization on pollinators, many studies conclude the negative impact of urbanization, such as habitat fragmentation, the introduction of invasive species, loss of resources and nesting site (Martins et al., 2017; Vanbergen & Initiative, 2013). This negative impact of urbanization correlates with the density of Hymenopteran species, especially bee species. Because many species and functional groups respond differently to the anthropogenic drivers such as land-use change, detecting the effect of urbanization on the community of the species is itself very challenging.

In this study, one single species of honeybee was recorded. Deguines et al. (2012) found to have a lesser impact on bees by urbanization. The study by Hennig & Ghazoul (2011) found a higher abundance of honeybees in urban areas. Still, the present study did not record any wild honeybees. The decline of the honeybee population due to many ecological and anthropogenic pressures such as pesticides, heavy pathogens and decreasing resources has been widely reported (Potts et al., 2010; Vanbergen & Initiative, 2013).

The diversity and the abundance of Diptera species were relatively higher as compared to other pollinating species. Non-bees such as Diptera are reported to respond less negatively to the land-use changes and have diverse nesting habits (Rader et al., 2015).

The Shannon Wiener Diversity Index of Lepidoptera was 1.87, Diptera was 1.69, Hymenoptera 0.9 and the least was Coleoptera (0.08) during the study period. Similarly, the species richness was 11, 7, 4, and 1, respectively (Fig. 2).

Shift wise distribution of pollinators

A total of 17 species of pollinators were recorded in the daytime, accounting for high species richness, whereas in the morning, five species, and evening only three species were recorded. The Diversity index was 2.63, 1.54, and 1.06 in daytime, morning and evening respectively (Fig. 3).

During the study period, the diurnal activity pattern of pollinator was highest at daytime (62.53 %) and least at evening (13.34%). In a day, Hymenoptera was active for 11.75%, Lepidoptera, 22.38%, Diptera, 61.57% and Coleoptera for 4.30% time of the total observation (Table 2). Species from order Diptera were observed in all three shifts, but Lepidoptera was not recorded during morning and evening, Hymenoptera was not recorded in the evening, and Coleoptera was not seen in the morning.

Table 1 List of pollinators recorded in the study area

S.N.	Scientific Name	Family	Order
1	*Brassicogethens aeneus*	Nitidulidae	Coleoptera
2	*Calliphora vomitaria*	Calliphoridae	Diptera
3	*Episyrphus balteatus*	Syrphidae	Diptera
4	*Episyrphus viridaureus*	Syrphidae	Diptera
5	*Eristalinus aeneus*	Syrphidae	Diptera
6	*Eristalis transversa*	Syrphidae	Diptera
8	*Pollenia rudis*	Calliphoridae	Diptera
9	*Musa domestica*	Muscidae	Diptera
10	*Polistes* spp	Vespidae	Hymenoptera
11	*Vespa velutina*	Vespidae	Hymenoptera
12	*Polistes dorsalis*	Vespidae	Hymenoptera
13	*Ariadne merione*	Nymphlidae	Lepidoptera
14	*Nyctemera adversata*	Eribidae	Lepidoptera
15	*Parnara guttata mangala*	Hesperiidae	Lepidoptera
16	*Pieris canidia indica*	Pieridae	Lepidoptera
17	*Pseudolus wedah*	Nymphalidae	Lepidoptera
17	*Euchrysops cnejus*	Lycaenidae	Lepidoptera
18	*Eurema blanda silhetana*	Pieridae	Lepidoptera
19	*Neptis ananta ananta*	Nymphalidae	Lepidoptera
20	*Algais cashmerensis*	Nymphalidae	Lepidoptera
21	*Vanessa cardui*	Nymphalidae	Lepidoptera

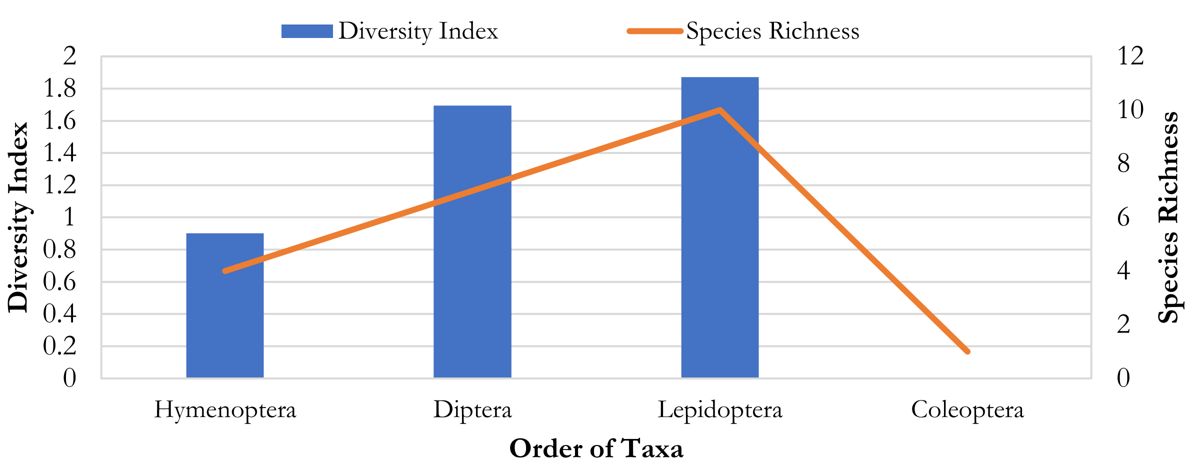

Figure 2 Shannon Wiener diversity index and species richness

The distribution of the pollinators in the marigold was varied at different times and shifts. The maximum number of pollinating species were observed during the daytime. A similar observation was reported by Rianti et al. (2010) in *Jatropha curcas L.*, where the high diversity of pollinators was recorded to be in day hours as compared to the morning and evening hours. The observation agrees with the study concluded by Kopelkievskii (1953), which stated that the insect visitors mostly forage on the flowers during the daytime between 0900 through 1200 hours of the day. The day hour is considered the peak hour for foraging activities for many pollinating species as the nectar flow is higher in flowers, particularly during the early hour of the day.

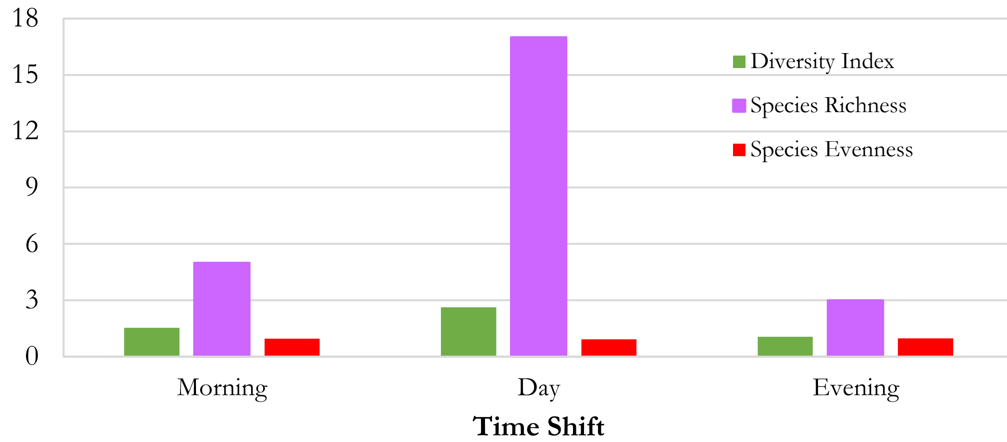

Figure 3 Shift wise distribution of pollinating species

Table 2 The relative species abundance of pollinating species in different shift

SN	Taxon	Day	Morning	Evening	total
1	Hymenoptera	8.90%	2.85%	-	11.75%
2	Lepidoptera	22.38%	-	-	22.38%
3	Diptera	29.20%	22.33%	10.04%	61.57%
4	Coleoptera	1.40%	-	2.90%	4.30%
	Total	61.88%	25.18%	12.94%	100.00%

Total visitation frequency

The highest foraging rate of three visits per minute during the daytime on a flower was recorded by five species, *Vespa velutina,* Diptera like *Calliphora vomitaria*, *Musca domestica,* and Lepidoptera like *Pieris canidia indica* and *Parnara guttata mangala,* as shown in (Fig. 4). Likewise, pollinating species such as Diptera like *Eristalis transversa, Episyrphus balteatus* and Lepidoptera such as *Eurema blanda silhetana, Neptis ananta ananta, Pseudolus wedah* recorded the least visit/minute with only one visits per minute. The Lepidoptera was the most abundant species after Diptera and had the highest diversity index and species richness. Among Lepidopteran species, *Pieris indica* and *Vanessa cardui* were the most abundant species in marigold, as Ganai et al. (2017) reported, which is similar to our study. Moreover, the diversity of butterflies is expected to depend on the availability of food plants and habitat quality, which implies that the urban areas might account for the quality foraging area for butterfly species as per the observance of the diversity of butterflies in our study. However, the study was done on the density and the diversity of butterflies in urban areas reported the species richness of butterfly species negatively correlated with urbanization (Lee et al., 2018).

Similarly, the study done by Dylewski et al. (2019) showed the butterfly species to be the most sensitive pollinator group in the urban green area such as parks and are particularly affected by human-induced habitat change and management practices. Our study contradicts the previous study carried out on the butterflies in urban areas. Likewise, there have been many studies regarding the effectiveness of pollination by butterflies. Barrios et al. (2016) argue in the research that despite the butterflies having higher abundance and visitation frequency, they contribute very little to the pollination of the flower. Many butterflies, such as skipper and non-skipper butterflies, were observed to act as nectar thieves as they did not carry much pollen in their proboscis nor deposited the pollen in the stigma (Barrios et al., 2016). However, some studies reported that butterflies are somewhat or completely effective pollinators in some plant species in the tropics (De Araújo et al., 2014).

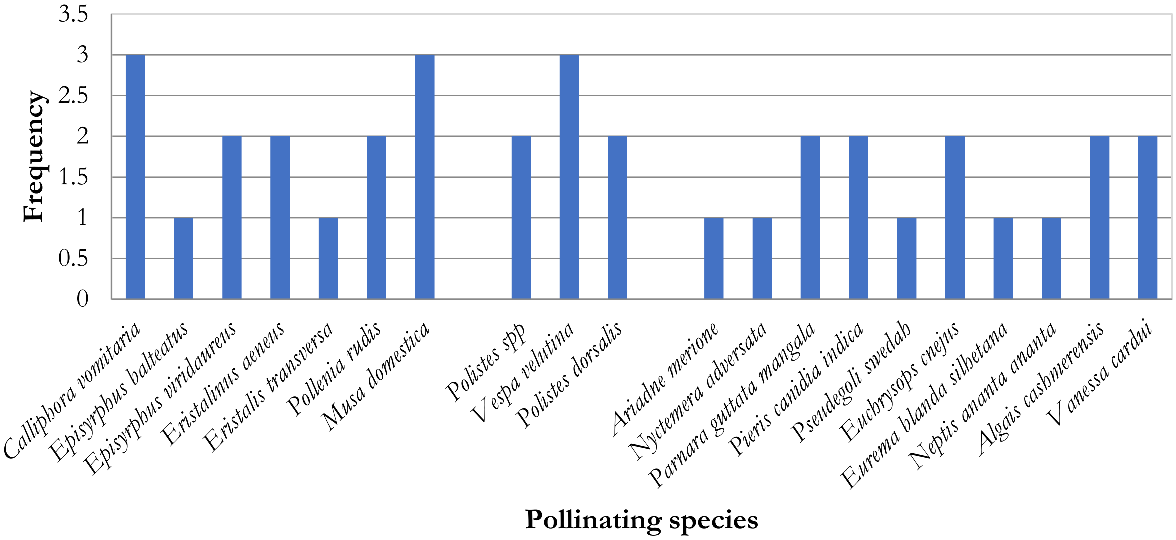

Figure 4 Visitation Frequency of each pollinating species in a marigold flower per minute

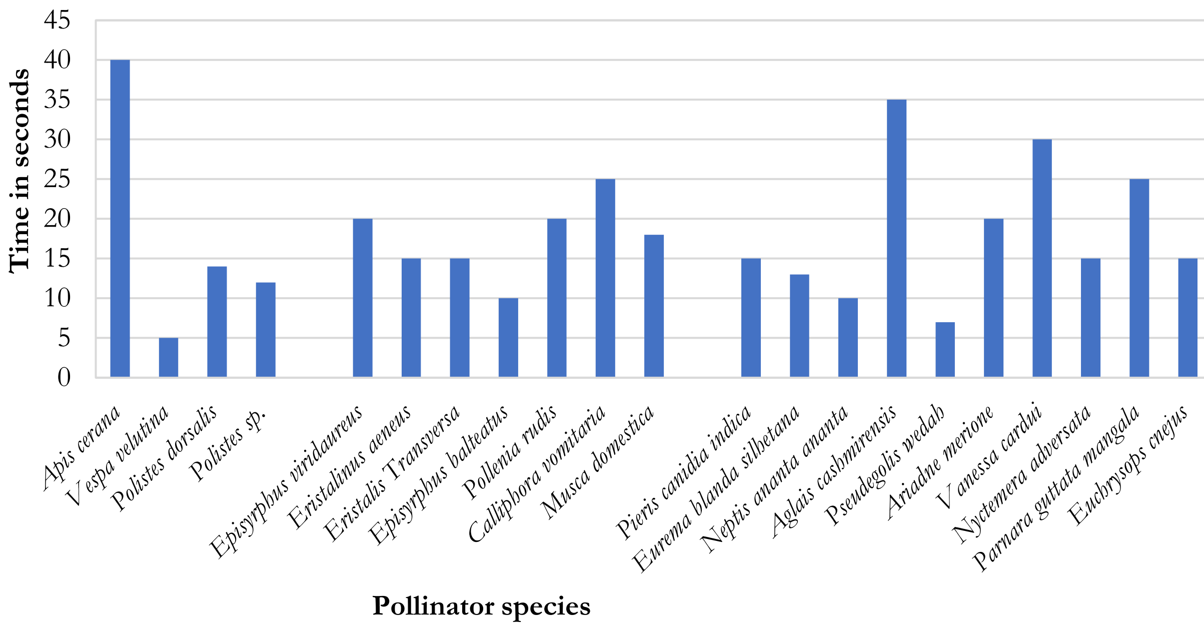

Figure 5 Time duration of the pollinating species in a marigold

Furthermore, Syrphidae were reportedly observed to be indiscriminate in the colour of flowers they visit. It is likely that the visitation of the flowers of various colours represents the foraging preference rather than the difference in the visual system (Klecka et al., 2018). They also observed that among the Dipteran species, *Episyrphus balteatus* from the family Syrphidae were the least selective species towards the colour, which supports our observation of high abundance of *Episyrphus balteatus* as compared to other species of Syrphidae in marigold. In addition, the study observed higher visitation of Syrphid in yellow flower, which further concludes the higher diversity and abundance of Syrhidae in marigold flower. Moreover, Syrphidae was recorded to have greater interaction evenness, which implies potential stability of the plant-pollinator community (Orford et al., 2015). They also revealed that the pollen load of the Syrphidae did not differ much from Hymenoptera, which strongly implies that the Dipteran groups play an important role as Hymenoptera for effective pollination. One of the studies done on the non-bee pollinators demonstrated the importance of non-bee pollinators and suggested that non-bee taxa can also make a substantial contribution to global pollination service (Rader et al., 2015).

Moreover, many studies argue that the Syrphidae are capable of providing 'insurance value' that fill the niche of declining bee species as the Dipteran population is considered to have similar functional attributes to Hymenoptera species such as mouthparts, feeding behaviour and phenology (Orford et al., 2015; Rader et al., 2015).

Among the 21 species, A*lgais cashmeresis* accounted for the highest time duration of average 35±2.05 sec per flower followed *Vanessa cardui* by 30±1.52 sec per flower and *Parnara gutata mangala* by 25±1.05 sec per flower. Pollinating species like *Vespa velutina* and *Pseudolus wedah* showed less amount of time in the flower of 5 second and 7 second, respectively. Moreover, Coleoptera was observed to spend longer time (Fig. 5). Likewise, one species of coleoptera i.e., Pollen beetle was found in a single flower and was observed to be less mobile. Many studies conducted in rape seed oil, identified pollen beetles as a pest (Szulc, 1963) as they lower the visitation efficiency by reducing the pollen availability and damage the plant by increasing food abortion (Alford et al., 2003).

Conclusion

In this study, a total of 21 species of pollinators from fourteen families and four orders, among them the Shannon Wiener Diversity Index of Lepidoptera was 1.87, Diptera was 1.69, Hymenoptera 0.9 and the least in Coleoptera (0.08) during the study period. Similarly, the species richness was 11, 7, 4 and 1, respectively. Marigold flowers were mostly visited by various pollinating species such as Hymenoptera, Diptera, Lepidoptera and Coleoptera. Among them, Lepidoptera accounted for the highest diversity index and species richness. Pollinators are active at daytime and less active or many species are absent in the morning and evening time shift. Absence of honeybee species in the study area creates an alarming situation for conservation of pollinating species in urban area. There is no policy and regulation for bee conservation in Nepal which is strongly demanded for their conservation that can lead to biodiversity conservation and growth in agricultural productions.

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Appendix – I Pictures of insect pollinators


Polistes dorsalis
Vanessa cardui
Eristalinus aeneus
Episyrphus viridaureus
Coleoptera
Nyctemera adversata