DIVERSITY OF INSECT POLLINATORS AND THEIR IMPACT ON THE CROP YIELD OF MUSTARD (*Brassica campestris* L.) IN KUSMA, PARBAT, NEPAL

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This is to recommend that the thesis entitled **"DIVERSITY OF INSECT POLLINATORS AND THEIR IMPACT ON THE CROP YIELD OF MUSTARD (*Brassica campestris* L.) IN KUSMA, PARBAT, NEPAL"** has been carried out by Mr. Narayan Subedi for the partial fulfillment of Master's Degree of Science in Zoology with special paper Entomology. This is his original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Mr. Narayan Subedi entitled **"DIVERSITY OF INSECT POLLINATORS AND THEIR IMPACT ON THE CROP YIELD OF MUSTARD (*Brassica campestris* L.) IN KUSMA, PARBAT, NEPAL"** has been accepted as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper Entomology.

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I hereby declare that the work presented in this thesis 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 reference to the author(s) or institution(s).

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ABSTRACT

Pollination is the most important ecosystem service provided by insects, resulting in sustainability and continuity of the ecosystem. The study was conducted to explore the diversity of insect pollinators and their impact on crop yield of mustard in Kusma, Parbat, Nepal. Four blocks each of size 12 m^2 were established by purposive sampling technique considering homogenous and continuous crop cover. Each block had two treatment plots i.e experimental and control plot. Insect diversity was observed in three phases of mustard blooming from 8 hr to 16 hr. Similarly, to find the impact of insect pollination on crop yield, randomly 10 mustard plants from each treatment plots were selected and tagged just before flowering. Finally, all the tagged plants were examined for various qualitative and quantitative parameters. The pollinator insects of mustard included 16 species under five different orders and nine families. Among them, Hymenoptera (36%) was the most abundant order visiting mustard flowers followed by Diptera (34%), Coleoptera (17%), Lepidoptera (12%) and the lowest Heteroptera (1%), whereas Apidae (35.64%) was most abundant family followed by Syrphidae (31.84%). *Apis cerana* and *Eristalis* sp. were most important pollinator insects of mustard. Among the recorded 16 species, seven species were found foraging on both pollen and nectar, four species foraging only nectar and remaining five as causal visitors only. The peak foraging activities of most of insects were observed between 12 hr to 14 hr. A significant differences in the abundance of pollinator insects were observed during different phases of flowering (p=0.001). A significant difference was observed in number of branches (4.050±0.171 & 5.025±0.180), number of pods (59.80±1.967 &70.47±2.431), fruit set (70.55±1.362 & 80.94±0.638), number of seeds per pods (16.70±0.248 &19.30±0.330), diameter of seed (0.133±0.2547 & 0.275±0.0051) and weight of 100 dry seeds (0.33±0.058 & 0.48±0.023) respectively in control and experimental plots, whereas, the difference was non- significant in case of height of plant, number of flowers and length of pod between control and experimental plot (p>0.05). Therefore, pollinator friendly cultivation practices should be followed for conservation and management of insect pollinators for higher production and productivity of mustard.

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LIST OF ABBREVATIONS

Abbreviated form	Details of Abbreviations
ANOVA	Analysis of variance
Asl	Above sea level
DAS	Days after sowing.
Df	Degree of freedom.
E	East
FAO	Food and agriculture organization.
IPBES	Intergovernmental Platform on Biodiversity and Ecosystem Services .
IPCC	Intergovernmental Panel on Climate Change
MEA	Millenium ecosystem assessment.
MoAC	Ministry of Agriculture and Co-operatives.
N	North.
No	Number
SD	Standard deviation.
SE	Standard error.
Sp.	Species.
S.N.	Serial number.
wt.	Weight.
%	Percentage.
&	and

1. INTRODUCTION

1.1 Background

1.1.1 Biodiversity and Ecosystem services

Biodiversity, the variety of life on earth, contributes both directly and indirectly to human welfare and existence on the planet by the provision of vital goods and services (Costanza *et al.,* 1997; Daily *et al.,* 1997; Kearns *et al.,* 1998; Palmer *et al.,* 2004). These can be termed "ecosystem services" and can be described as "the benefits to human welfare provided by organisms interacting in ecosystems" (Hooper *et al.,* 2005; Klein *et al.,* 2007), or the "economic benefits that nature provides to people" (MEA, 2005). In more recent times, both biodiversity and the ecosystem services it provides are under increasing pressure from human activities (Daily *et al.,* 1997; Vitousek *et al.,* 1997; Hooper *et al.,* 2005; Cardinale *et al.,* 2012). However, humans and biodiversity are not separate entities, and the maintenance of biodiversity and ecosystem services globally is increasingly dependent on maintaining biodiversity in landscapes dominated by humans (Fahrig *et al.,* 2011).

1.1.2 Pollination and its importance as an ecosystem service

Agricultural production forms one of the most important economic sectors where the quality of most crop species is increased by pollination (Klein *et al.,* 2007; Gallai *et al.,* 2009). Pollination is an important process in maintaining healthy and bio diverse ecosystem. Pollination not only improves the yield of the crop, but it also contributes to uniform and early pod setting (Abrol, 2007). Pollination is an essential supporting ecosystem service required by the majority of flowering plants; it has been estimated that 87.5% of angiosperms require biotic pollination (Ollerton *et al.,* 2011), and that 62% of these flowering species are limited in reproduction by the amount of pollen they receive (Burd, 1994). Pollination is the result of pollen being transferred from the anther (male part) to the stigma (female part) of another flower. Although this can happen by abiotic means (via transport in water or by wind) the majority takes place through transport on the bodies of flower visiting animals. A wide variety of organisms can act as pollinators including birds, bats, other mammals and insects (Willmer *et al*., 1994), with insects being the most common. Pollinators are not only responsible for the reproduction of wild plant species, but also for pollination of a large number of food crops for humans (Corbet *et al.,* 1991). Thirty nine of 57 leading world food crops have higher production with pollination, with 35% of the world's food supply coming from insect pollinated crops (Klein *et al.,* 2007). Pollinator dependent crops also provide many essential nutrients required for a balanced human diet (Eilers *et al.,* 2011), and are increasing in production (Aizen *et al.,* 2008) and price (Lautenbach *et al.,* 2012) globally.

1.1.3 Biology and economic importance of mustard

Mustard is one of the important cash crops of Nepal, which occupied 233041 ha area, with production of 209612 mt and productivity of 0.89 mt/ha in 2014/2015, where as in 2015/2016, area decreased to 217867 ha, with production of 208291 mt and productivity of 0.95 mt/ha (MoAC, 2015/2016). According to FAO, the estimated total commercial production of mustard seed (of all species) was 59.1 million metric tons worldwide, harvested from 31.7 million hectares. Canada and Nepal were the leading producers, together accounting for 321 thousand metric tons, followed by the Ukraine, Myanmar, the Russian Federation, and the U.S. (FAOSTAT, 2013). Other oilseed crops grown in the country are soybean, sunflower, sesame, groundnut, castor, linseed, and niger. Mustard alone occupies about 85% of the total oilseed area in the country and it is a dominant winter season oilseed crop (Basnet, 2005), and it is the second largest oil seed crop that plays a vital role to sustain the human consumption of edible oil It is mostly grown after monsoon maize in upland and after early rice in lowland of Terai, inner Terai and mid-hills (Ghimire *et al.*, 2000).

Brassica campestris L. belongs to the Cruciferae (Brassicaceae) family, also known as the mustard family. It has green foliage, leaves glabrous or slightly hispid when young, and the upper leaves partially clasping the stem. The stems are well branched, although the degree of branching depends on biotype or variety and environmental conditions. Branches originate in the axils of the highest leaves on the stem, and each terminates in an inflorescence. Lower leaves are sparingly toothed or pinnatifid and petioled, while upper leaves are sessile, subentire, oblong lanceolate and often constricted above the base. The inflorescence is an elongated raceme, the flowers are pale yellow, densely clustered at the top with open flowers borne at or above the level of terminal buds, and open upwards from the base of the raceme (Downey *et al.*, 1980). Flowers of mustard are formed in the stem elongates after the leaf stop growing. The stem has many branches, small leaves, and bright yellow flower. Each flower has four petals and six stamens of which two stamens are shorter than the style but four others are longer. There is a single stigma at the tip of the style. Nectar is excreted at the bases of the short stamens and ovary. The flower is open for three days.

This crop is important from income generation point of view and is prominent sources of fats, protein and vitamins as compared to cereals and legumes in Nepalese diet (Chaudhary, 2001). Its seeds contain 40-45% oil and 20-25% protein (Hasanuzzaman *et al*., 2008). Similarly, 4.8% nitrogen, 2% phosphorus and 1.3% potash can be obtained from mustard oil cake (Prasai and Yadhav, 1999). Apart from this, its oil is also used for treating remedies like stomach ache, bone ache, muscle pains, skin disorders, etc. It has several anti-nutritional factors like eruic acid and eusinic acid. Mustard oil has a specific gravity of 0.90. The iodine value ranges from 87 to 122 and saponification value between 172 and 200. It is an excellent cooking oil as it contains high amount of oleic acid and palmetic acid and low amount of linolenic acid.

1.1.4 Insect pollination in Mustard

Mustard crop has a mixed pollination system, but is mainly self-fertile (Steffan-Dewenter, 2003). Considerable outcrossing has been observed (Olsson, 1960), and the degree has been shown to vary with environmental conditions and cultivars (Olsson, 1960; Becker *et al.*, 1992). Outcrossing can be mediated by wind, insects, or movements among plants, but their relative importance is unknown (Free, 1993). Mustard flowers are highly attractive to pollen and nectar feeding insects due to its bilateral, bright yellow flowers that produces nectar from four nectary glands situated in the bottom of the flower (Abrol, 2007). The pollen grains are sticky and aggregated, which is typical for insect pollinated plants (Cresswell *et al*., 2004). The flowers have a stigma surrounded by six stamens; two shorter that release pollen below the sigma, and four long stamens that first release pollen away from the stigma, but at the end of the flowering bend in-ward the flower. In this way, cross-pollination is favoured, but self-pollination can assure pollination at late flowering (Eisikowitch, 1981; Free, 1993; Abrol, 2007). Mustard flower attracts a wide range of insect species (Stanley *et al*., 2013).Insects constitute one among the primary groups of pollinating agents, as the association between insects and flowers are well established. Insect pollination is important to the reproduction and persistence of many wild plants (Ollerton *et al.,* 2011). Various insect groups, which are of prime significance in pollination of different agricultural, horticultural and medicinal herbal crops mainly belong to the orders Hymenoptera, Diptera, Coleoptera, Lepidoptera, Thysanoptera, Hemiptera and Neuroptera (Free, 1993; Kearns *et al.,* 1998 ; Mitra and Parui, 2002; Mitra *et al.,* 2008).

Mustard is cross-pollinated crop and requires sufficient pollinating agents for better pollination and seed production. The yellow color of the flower with shallow placement of visible nectar mostly attracts bees, flies, and butterflies. Honey bees have also been documented as the most frequent visitor of mustard flowers (Free and Nuttall, 1968). Honeybees visit rapeseed flowers for collection of both pollen and nectar, which in turn results into florets cross-pollination Honeybees are most important pollinating insect (Williams, 1994; Sharma *et al.,* 2004). The main significance of honeybees and beekeeping is pollination, whereas the hive products (honey, wax etc.) are of secondary value (Verma, 1990). Out of total pollination activities, over 80% is performed by insects and bees contribute nearly 80% of the total insect pollination and therefore, they are considered the best pollinators (Robinson and Morse, 1989). The yield of oilseed rape is tightly associated with the sort and amount of pollinators. The insect pollination could increase seed yield and quality in oilseed rape. As early as 1981, bumble bees and honeybees were the main visitors of rapeseed (Eisikowitch, 1981). It is reported that the yield and quality of rapeseed increased significantly by pollination by honeybees and other insect pollinators. Therefore insect pollination is the most economic and efficient way to increase the yield of rapeseed (Tara and Sharma, 2010; Sushi *et al.*, 2013). Several field investigations have reported that honeybee pollination on rape seed significantly elevated the pod set and productivity, which hymenopteran insects account for 92.3% of all the visiting insects, and 99.8% of which are *Apis mellifera* (Annelise *et al.*, 2011; Shakeel and Inayatullah, 2013). Bumblee bee being the important pollinators of many agriculture crops, however constitute only 2% of all the insect pollinators in mustard crops.

1.2 Objectives

1.2.1 General Objective

To explore the diversity of pollinator insects in mustard and their impact on crop yield of mustard.

1.2.2 Specific Objectives

1. To explore the diversity of insect pollinators and their relative abundance in mustard.
2. To examine the foraging sources and activities of insects during mustard flowering.
3. To analyse the effects of insect pollination on crop yield of mustard.

1.3. Rationale of research

Pollination by insects has been identified as an important ecosystem service. Despite this, there is lack of up to date studies on pollination of crops and there is surprisingly little information available on the extent to which insects contribute to crop seed set via pollination. Low pollinator abundance and diversity have also started appearing in different parts of the world. Due to continuous use of pesticides and declining of natural habitats, insect pollinators are declining rapidly. Despite the importance for human edible oil consumption and diversity of eco-type in Nepal, information regarding to the abundance and diversity of pollinating insects on rape seeds has not been reported much. Diversity and abundance of wild insect pollinators play crucial roles in crop pollination, particularly for insect-pollinated crops. High levels of biodiversity are vital for enhancement of ecosystem function via interspecific facilitation. Conservation of pollinator's diversity is crucial to food production and the diversity of wild plants. The large-scale agriculture is reported to reduce the diversity and abundance of wild insect pollinators in many agricultural landscapes. Knowledge of specific pollinators of mustard crops is limited in context of Nepal although its pollination requirements have been studied in other countries. Therefore, this study was carried out to identify insect pollinators of mustard and their impact on crop yield.

2. LITERATURE REVIEW

2.1 Insect pollination in crops

The loss of biodiversity caused by human activities is considered to exceed the thresholds for a stable environmental state on Earth (Rockstrom *et al.*, 2009). When species interact with their environment in an ecosystem, ecological processes occur. Some of these ecological processes provide services that benefit humans (Cardinale *et al.*, 2012), which are defined as ecosystem services (Daily, 1997; MEA, 2005). One often highlighted ecosystem service as insect pollination. The loss of biodiversity among pollinators has raised questions about whether the pollination services they provide are at risk (Garibaldi *et al.*, 2011). The Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES) was initiated in 2012 to provide governments and society with independent and scientifically based assessments of biodiversity and ecosystem services, corresponding to the Intergovernmental Panel on Climate Change (IPCC). IPBES has recently compiled a thorough review of the scientific literature and assessed the global status of pollinators, pollination and food production (IPBES, 2016), and concluded that pollinators and the pollination service they provide are threatened by land-use change, agricultural intensification, climate change, pesticide use, pathogens, genetically modified organisms, and invasive species (Dicks *et al.*, 2016; IPBES, 2016; Potts *et al.*, 2016). Pollinators play key role in the survival of integrity of terrestrial ecosystem through their major role in plant reproduction, thereby providing services and goods to the society. Pollination by insects and other animals is significant in most terrestrial habitats. Fruit, vegetable or seed production from 87 of the 115 leading global food crops depends upon animal pollination. It involves 67% of species of flowering plants and a relatively high diversity of insect taxa (Sree Latha *et al.*, 2018). On the other hand, 35% of crop production worldwide and 70% of major global crop species rely on animal pollination. (Lautenbach *et al.*, 2012) estimated values of bee pollination at €265 billion in global food supply. The dependence on pollination varies significantly among crops, with some crops having increased yields up to 100%, while others having increases of just a few percent (Chiari *et al.*, 2005).

Kumar and Naidu (2010) estimate about 1200 species of vertebrate pollinators and 80,000 species of insect pollinators are present worldwide. Amongst 80,000 species of insect pollinators, 17,533 species are of bee pollinators worldwide, out of these 633 species from 60 genera belongs to 6 families were reported from India. Among the insect pollinators, honey bees are the most important pollinators of angiosperms because of their vegetarian diet, flower visiting habits, floral fidelity, presence of thousands of work force, large number of hairs on body that readily pick up pollen grains and the fact that they exclusively visit many flowers of the same species during a single trip and also their availability throughout the year makes honey bees the most efficient and reliable pollinator. Honeybees pollinate 16% of the total of 0.25 million of flowering plant species in the world and nearly 40000 species of agricultural plants. Worldwide, 90 per cent food supply is contributed by 82 commodities assigned to plant species and bees are pollinators of 63 (i.e. 70%) of these plant species and are the most important known pollinators of 39 (48%) of these plant species. One-third of human diet is derived directly or indirectly from bee pollination in developed countries. Of the hundred or so animal-pollinated crops which make up most of the world's food supply, at least 80 % are pollinated by honey bees and wild bees (Waykar and Baviskar, 2015).

2.2 Diversity of insects visiting flowers and their abundance

Devi *et al.* (2017) studied the diversity of insect visitors on mustard at Dr. Y.S. Parmar University of Horticulture and Forestry. A total of 88 insects belonging to 63 genera under 31 families and nine orders were found to visit mustard bloom. Hymenoptera was the most abundant order with 12 families, Diptera was the second most dominant order with four families followed by Lepidoptera with five families. Bhowmik *et al.* (2014) carried out study from South Bengal region to explore the diversity of insect pollinators of *Brassica juncea* and their influence on seed yield and quality. They recorded 19 different insect species under four orders. Among the four orders, Lepidoptera and Diptera shared maximum number of species (six and seven species), followed by Hymenoptera (four species) and Coleoptera (two species).
Goswami and Khan (2014) studied diversity and abundance of different insect visitors on mustard at Pantnagar. A total of 19 insect visitors belonging to order Hymenoptera (15) and Diptera (four) were found to visit the mustard blossoms. The abundance of Hymenopterans was maximum followed by Dipterans and others. In Hymenopterans, the honey bee (Apis bees) were observed maximum followed by non-Apis bees and the scolid wasp. Soliman *et al.* (2015) conducted a study on insect pollinators diversity and their impact on yield production of Canola (*Brassica napus*) in Ismailia, Egypt. The result revealed that 21 species of insect pollinators belonging to 14 families under four orders visited canola flowers. The abundance of Hymenoptera insects reached the maximum of 67.90%, followed by Diptera 14.97%, Coleoptera 13.61% and Lepiodoptera 2.26% as average of both seasons.

Pudasaini *et al.* (2015) studied the abundance and diversity of rapeseed insect flower visitors at Jutpani VDC, Chitwan district. The pollinator fauna of rapeseed included 21 species from six different insect orders. Hymenopterans (77.95%) were the most abundant insect visiting rapeseed followed by Dipterans (12.23%) and Lepidopterans (3.49%). Honey bees were the most dominant group of pollinators. Shakeel and Inayatullah (2013) recorded nine species of pollinator insects in canola, among which *A.mellifera, A. florea, A. dorsata, and A. cerana* were the major pollinators. A total 14 bee species from nine genera were identified as pollinators, found on different crops at NIPHM. Out of 14 species of bees, five bee species were honeybees from family Apidae and Genus *Apis* and *Tetragonula*, and remaining nine species were solitary bees (Sree Latha *et al.*, 2018). Dhakal (2003) and Atmowidi *et al*. (2007) listed four orders of insect namely: Hymenoptera, Lepidoptera, Diptera and Coleoptera occurring in rapeseed field. Similarly, Rader (2010) observed five insect orders, namely: Hymenoptera, Lepidoptera, Diptera, Coleoptera and Hemiptera in rapeseed field. Kunjwal *et al.* (2014) observed a total of 30 species belonging to four orders Hymenoptera, Diptera, Lepidoptera and Coleopteran visiting mustard, *B. juncea* flowers. Among them, Hymenoptera were the major insect pollinators. It was also observed that *A. mellifera* was most abundant species in all the varieties of *B. juncea* than other bees. Kamel *et al.* (2015) observed 21 species of insect pollinators belonging to 14 families under four orders visiting canola, *B. napus* flowers. Ahmad (2005) reported that 22 and 16 Hymenopterans and 7 and 5 Dipterans species visiting mustard flowers in Diriyah and Derab (Saudi Arabia), respectively. They observed honey bees as the dominant Hymenoptera pollinators followed by other bees such as *Andrena, Hexachysis, Osmia, Pompilus*, Dieles and Wasps.

Panda *et al.* (1989) reported that mustard flowers were visited by seven species of insects, of which four species belonged to family Apidae, two to Anthophoridae and one species to Andrenidae. Langridge and Goodman (1975) reported that the oil seed rape crop was visited by many insect species. Of which honeybees were major visitors and accounted about 32.9% followed by hoverflies (30.7%), bowflies (22.9%), native bees (4.9%) and others (8.8%). Radehenko (1964) reported that honey bee constitute 97.2% of all pollinators during full bloom of winter rape, and 94.7% at the beginning and 88% at the end of blooming. The rest species were of Andrenidae, Halictidae and Bombicidae. Sharma *et al*. (1974) investigated on flower visitors of cauliflower and found that 34 insects species belonging to 23 families falling under five orders visited the bloom. Out of these, *Apis cerana* (42.1%), *Eristalis* spp. (20.9%), *Ceratina* spp. (15.1%), *Halictus* spp. (5.3%), *Lasioglossum* spp. (4.7%), *Xylocopa* spp. (3.2%), *Apis dorsata* (2.9%) and *Bombus haemorrhoidalis* (1.9%) were important pollinators. Kapil *et al.* (1971) found *A. florea* to be principal visitor of mustard *(Brassica juncea*) and their number during 1966-67 varied from 82.89 to 204.73 bees/100 sweeps. The other species associated with this crops were *Andrena ilerda, Apis dorsata* and halictine bees. Varma and Joshi (1983) found that *Apis cerana* and other bees accounted for 58.7% of the total pollinators of mustard. Of the honey bees *Apis cerana* was 49.9%, *Apis dorsata* 22.43% and *Apis florea* 27.57%. Kumar *et al.* (1988) found that among all insects visiting some cultivators of cauliflower, *Apis cerana* was the most frequent visitor (38.75%) followed by Dipterans (29.74%) and other Hymenoptera (11.86%). The least frequent visitor was *Apis mellifera*.

2.3 Foraging sources and activities of pollinator insects

Ali *et al.* (2011) found that the Nectar was the primary foraging task of all the bee species except *Halictus* spp., which also fed on pollen. In contrast, no fly species fed on nectar alone. Four species *(Episyrphus balteatus, Melanostoma* spp.*, Euopeodes corollae,* and *I. scutellaris) of* Syrphinae (Syrphidae*)* fed solely on pollen, and the other two species *(E. laetus* and *E. aeneus)* of Eristalinae (Syrphidae*)* and members of the other three Diptera families fed on both nectar and pollen. A single butterfly species *(D. chrysippus)* foraged exclusively on nectar.
Roy *et al.* (2014) carried out study at agricultural land of Phaldi, near Duttapukur, West Bengal to document the diurnal insect diversity, their activities, roles and abundance in

mustard flower. Twenty four insect species belonging to 14 families and six orders were found at day time in mustard blooms among which four species (*Apis dorsata, Apis cerena, Apis florea, Vespa* sp.) were pollen and nectar collectors, 13 species were only nectar collector and rest species were only visitors.

Pudasaini and Thapa (2014) studied the foraging behavior of *Apis mellifera* L. and *Apis cerana* F. in rapeseed flower under open and cage conditions in Chitwan, Nepal during 2012-2013. This study showed that both species of honeybee forage higher number of flower under open condition as compare to cage. The peak foraging hours for both species was around 12:00 pm to 14:00 pm. Peaks of foraging activity of both *C. lacunatus* and *A. mellifera* were mainly observed from 1:00 to 3:00 pm and they corresponded to the number of flowering plants (Soliman *et al.,* 2015). Kapil *et al.* (1971) showed that *Apis dorsata, Apis florea, and Apis cerana* begin foraging from 7 hr to 17 hr on mustard. Foraging reached a peak between 12. 30 hr to 14 hr and ceased by 17 hr. Rana *et al.* (1977) indicated that the mean number of *Apis cerana* foragers (1.90/m^2) was significantly higher than the *Apis mellifera* foragers (0.54/m^2) on rapeseed bloom. In both species the total foraging activity was significantly higher at 12 hr (0.91/m^2 of *Apis cerana* and 3.16/m^2 of *Apis mellifera*) than at 09 hr (0.16/m^2 of *Apis mellifera* and 0.55/m^2 of *Apis cerana*). However, in both the species there were no significant difference in the population of bees between 12 to 15 hr. Sihag and Khatkar (1999) observed that *Apis dorsata* initiated foraging on mustard flowers at 9 hr and reached peak at 12 hr and then the population declined. But in case of *Apis mellifera* and *Apis florea* started appearing on flowers around 10 hr and reached peak at 13 hr.

2.4 Effect of insect pollination on yield of crops

Mustard yield is determined by four important yield components i.e plants per area, number of siliques per plant, number of seeds per silique, and seed weight (Habekotte, 1993; Diepenbrock, 2000). It has a high plasticity and can combine these components in various ways to produce high number of seeds. Seed number per area is determined during flowering and is regarded as a main factor for increasing yield (Diepenbrock, 2000; Gomez and Miralles, 2011).

Mishra *et al.* (1988) revealed that percent pod setting, seed weight, seeds per pod and proportion of healthy seeds were significantly higher in open pollinated flowers then in the net caged and muslin bagged ones. Panda *et al.* (1989) reported that insect pollinated mustard crop had higher pod set (71.90%), average seed number per pod (10.80), 1000 seed weight (500gm), seed yield per ha (13.90 qt) and oil contents (36.40%) compared to the crops that was excluded from the pollinators. Kumar *et al.* (1988) showed that modes of pollination did not affect the pod formation in different cultivars of cauliflower but the number of seeds per pods and seed weight was greatly affected. Pudasaini and Thapa (2014) conducted an experiment on mustard to study the effect of pollination on its production in Chitwan during 2012-2013. Seed set increased by 48.72% with *Apis cerana* F. and 45.73% with *Apis mellifera* L. pollination as compared to the control. The highest seed yield was obtained from *Apis cerana* F. (1.11 mt/ha), followed by *Apis mellifera* L. (0.88 mt/ha), hand (0.75 mt/ha), natural pollination (0.66 mt/ha) and control (0.13 mt/ha), respectively. Prasad *et al.* (1989) reported that the plots which remained totally free from insect pollination had the lowest setting of siliqua (48.3%), which attained siliqua length (92.65mm) as compared to other treatments. The plots having free access to all the pollinators showed maximum siliqua setting (75.8%), which was closely followed by plots having honey bee (72.3%). Open pollinated plots had the maximum number of seeds per siliqua (10.58) and had the highest weight of 1000 dry seeds.

Sharma and Abrol (2014) found that open pollination resulted in 1.80 times more yield compared to caged condition and crop pollinated by bees alone on mustard crop. Goswami and Khan (2014) study the impact of different mode of pollination and found that highest percent pod set was in open pollinated (83.42%) plots followed by bee pollinated (75.41%) and caged pollinated (62.80%).The number of individual pollinators affected seed set of *B. juncea*. Seed set and abundance of pollinator insects was positively related. Pradhan (2003) reported that highest number of pods per plant 495 in *A. mellifera* pollinated plants, 438 in *A. cerana* and 417 in open pollinated plants whereas caged plants without pollinators produced 290 pods per plant. Kumar and Lenin (2000) found highest yield of *B. juncea* in open pollinated plots, followed by the plots caged with honeybees, whereas the caged plots (excluding pollinators) yield the lowest. Free and Nutall (1968) observed that *B juncea* plants caged with bees produced 25 per cent more seed than plants caged without bees. Highest seed set, seed per siliqua and 1000 seed weight was obtained by open pollination followed by hand pollination.
The impact of different mode of pollination found that significantly highest percent seed set was in open pollination followed by hand pollination and the minimum seed set was observed in pollination exclusion during two years of survey (Devi *et al.*, 2017). They also recorded that seed per siliqua and 1000 seed weight were significantly higher in open pollination followed by hand pollination and significantly less seed per siliqua was recorded in pollinators exclusion. Soliman *et al.* (2015) found that Open pollination increased the number of pods per plant, seeds per pod, weight of 1000 seeds, yield per plant, yield per feddan and seed germination, compared to close pollination.

Ali *et al.* (2011) found that with the increase in the number of seeds per pod, seed weight per pod also increased conforming the importance of insect pollinators in canola production. Varma and Joshi (1983) observed that pollination by honey bees increased the percent of pod setting by 44.70 and 74.20, number of seeds per pods by 2.32 and 4.07 and the weight of single seed by 0.19 mg and 1.17 mg in comparison to pollination by other insects and control pollination respectively. Langridge and Goodman (1975) observed that in oil seed rape, open pollinated plot produced 50% more seeds per plant and 46% greater weight of seeds per plant than plots from which insects were excluded. The weight of 1000 seeds was significantly greater in the pollinated plot than in the closed one. Khan and Chaudhary (1988) reported that self -pollinated sarson plants in Pakistan produced 20.46 to 34.34 gm seed per plant while honey bee (*Apis cerana*) pollinated and plants pollinated by other insects yielded 67.2 to 90.02 and 41.72 to 56.35 gm/plant respectively. Sinha and Chakrabarti (1985) showed that the plants that were left for open pollination gave 69.30%

and 64% more pods on cauliflower Cv.328 and Cv. Pusa Deepali, respectively than caged plants.

3. MATERIALS AND METHODS

3.1 Study Area

3.1.1 Location

The study was conducted in Agriculture land cultivated with mustard at Thulipokhari-12 Kusma, Parbat district from December 2018 to April 2019. It is a part of Gandaki Province. Its geographical location is $83^{0}42$’E longitude and $28^{0}11$’N latitude. This region covers mid hills area which is 1449 m asl.

3.1.2 Geomorphology and climate of Parbat

Parbat is a hilly district of Western, Nepal (Figure. 1). It is situated between 27° 28’ N to 28° 39’ N latitude and 83° 34’ E to 83° 59’ E longitude. The altitude varies from 520 m to 3,300 m whereas the annual rainfall is 2400 mm to 2600 mm. It has subtropical climatic zone.

The maximum temperature in summer exceeds 32.3°C and the average winter temperature is about 7.5°C. The soils are medium to high in organic matter and are hardly suitable for agriculture in many areas. Land-slides and soil erosion are severe in Parbat district during rainy season. The total area of Parbat district is 53,668 ha, out of which agriculture land, grazing/pasture land, forest land cover 16.8%, 28.22%, 37.25%, respectively and other land types cover 17.73%. Major forest types in this district are hill *Shorea robusta* forest, *Schima-Castanopsis* forest, *Pinus roxburghii* forest and *Quercus* spp. forest.

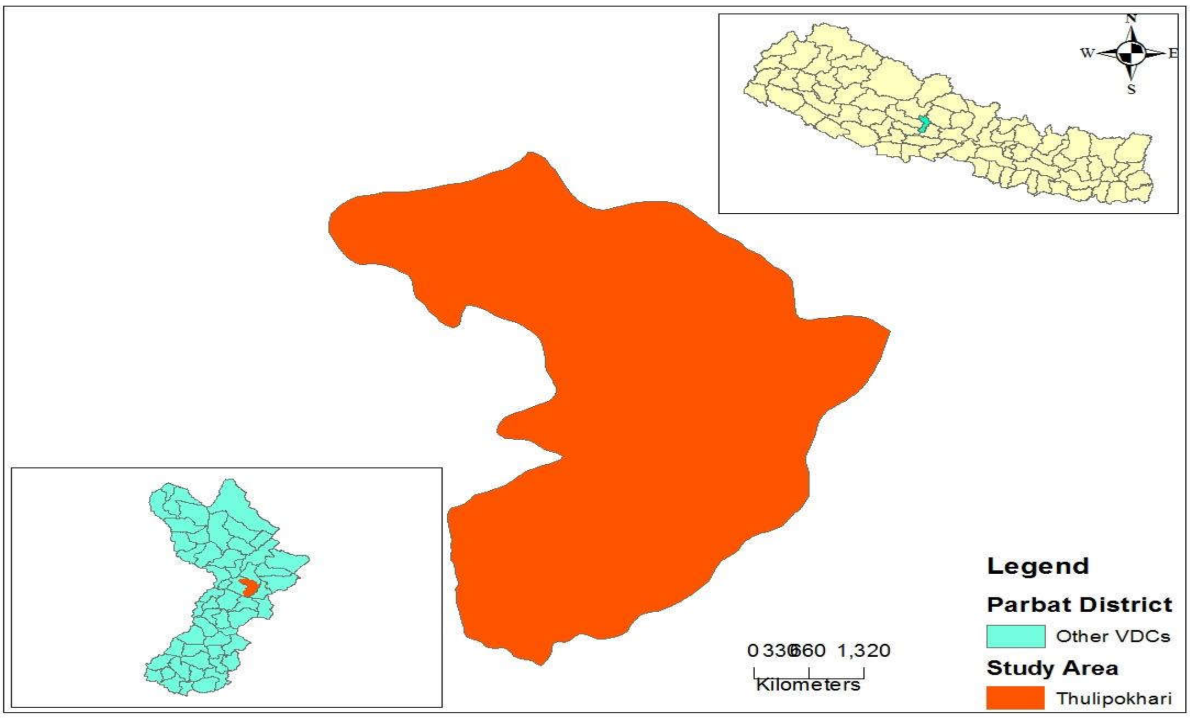


Figure 1. Map of Nepal highlighting Parbat district

3.2 Materials

3.2.1 Physical requirements

Insect trapping net, killing jar, yellow pan trap, camel hair brush, polythene bags, tracing paper electrical balance, measuring tape, vials, dropper, microscope, entomological pins, mosquito net, plastic rope, etc.

3.2.2 Chemical requirements

Formalin, ethyl acetate, ethanol, glycerol, camphor etc.

3.3 Research Methods

3.3.1 Experimental design

The field work was conducted in agriculture field sown with mustard. Within mustard field, four blocks each of size 12 m^2 were established by purposive sampling technique considering homogenous and continuous crop cover. Each block had two plots, and the size of each plot treatment was 3 m×2 m. Each block had two treatment plots, one experimental plot that was left open so that flowers were accessible for self, wind and insect pollination and another control plot that was enclosed by mosquito net of mesh size 1.2 mm so that the flowers were accessible to self and wind pollination but not insect pollination. Because it has been found that such nets do not hinder the deposition of pollen carried by wind, the difference between those two treatments represented the contribution from insect pollination in experimental treatment. The nets were kept before the onset of flowering and were left until the end of flowering.

3.3.2 Observation, Collection and Preservation of Specimens

Pollinator insects diversity were observed from 8:00 hr to 16:00 hr after the onset of flowering for three consecutive months (Jan- Mar) at the interval of every week. Four spots of 1 m^2 area were selected randomly and the number of pollinators visiting each square meter area were counted for two minutes for each period as adopted by Soliman *et al.* (2015). The specimens were collected using sweeping net and yellow pan traps was also used for trapping insects. Insect diversity was observed in three phases of mustard blooming i.e. early phase (about 10% flowering), peak phase (almost 100% flowering) and late phase (about 10% flowering remaining). The collected specimens were killed in killing jar containing ethyl acetate and was preserved in 90% alcohol. Butterflies were caught using sweeping net and were pinched in thorax and were kept in envelope made of tracing paper. The specimens were brought to Central Department of Zoology, Entomology laboratory where setting and pinning of specimens was done for further identification.

3.3.3 Analysis for crop yield of mustard.

Similarly, to find the impact of insect pollination on crop yield, randomly 10 mustard plants from each treatment plots from each block were selected and tagged them just before flowering. After the completion of flowering all nets were removed and tagged plants were left open in the field to ripen. Finally, all the plants from each plot were examined for various qualitative and quantitative parameters listed below before the farmer thresh the field.

a. Height of mustard plant.
b. Number of branches per plant.
c. Number of flower per plant
d. Number of pods per plant
e. Percentage of fruit set.
f. Length of pod.
g. Number of seeds in each pod.
h. Diameter of seed.
i. Mean weight of 100 dry seeds.

To estimate the number of flowers per plant, during each visit to a field the number of open flowers were counted from each of tagged plants from each treatment from all block. The last (topmost) open flower was marked with a colored plastic tag at each visit to keep track of the flowers that had already been counted. This process was continued until the end of flowering.

To estimate the effect of pollination on number of seed per pods, number of seed per pod was counted before harvest and to find the effect of pollination on fruit set following formula was used.

Fruit set =\{No. of pods (siliqua)/ no. of flowers\}×100% (Devi *et al.*, 2017).

3.3.4. Identification

Pollinator insects were identified with the help of Thapa (2015), Borrer and De Long (1964), Richards and Davies (1977) butterfly species were identified with Smith (2006). Also identification was done by sending picture of specimens to expert entomologists via social sites.

3.4 Statistical analysis.

All data were subjected to statistical analysis to compare the abundance and diversity of pollinator insects in different times of day and different phases of flowering.

A. Relative abundance (%)

Relative abundance is the percent composition of an organism of a particular kind relative to the total number of organisms in the area. Relative abundance was used to show the family-wise and order-wise composition of pollinator insects.

Relative abundance (%) = $(n/N) \times 100$

Where,
n= Number of each individual
N= Total number of individual

B. Shannon-Wiener diversity index (H) & Evenness index (J)

The diversity of pollinator insect species was calculated using Shannon-Winner diversity index (H), given by the equation, $H = -\sum pi \times \ln(pi)$.
Where, $pi = ni / N$, ni is the number of individuals of the species and $N = \sum ni$.
ln = the natural log
$\sum$ = the sum of calculations

To find the evenness of species pielou's species evenness index (J) given by, $J = H/H_{max}$ was calculated.
Where, $H = -\sum pi \times \ln (pi)$
$H_{max} = \ln (n)$, n is the total species richness.

C.ANOVA

Data obtained in the present study were subjected to analysis of variance (ANOVA) with the honestly significant value calculated as Tukey's statistic at $p<0.05$.
Linear mixed effect model was performed to compare the means between control and experimental plot for various qualitative and quantitative parameters of mustard plant for crop yield. For this purpose insect pollination effect was tested on each of the dependent variables, including block as random factors in mixed models.

D. Correlation

Spearman's Correlation was performed to find relation between length of pod and number of seeds per pod between control and experimental plot.

4. RESULTS

4.1 Diversity of insect pollinators

The present study recorded 16 pollinator insect species belonging to five insect orders (Hymenoptera, Diptera, Lepidotera, Coleoptera and Heteroptera) and nine families (Apidae, Bombidae, Syrphidae, Muscidae, Nymphalidae, Pieridae, Lycaenidae, Coccinellidae and Pentatomidae) (Table 1).

4.2. Species diversity and evenness index

The Shannon-Winner diversity index (*H*) was 2.2283 which indicates high diversity of pollinator insects in mustard field, with Pielou's species evenness (*J*) was 0.803689 (Appendix I).

4.3. Relative abundance of Pollinator insects

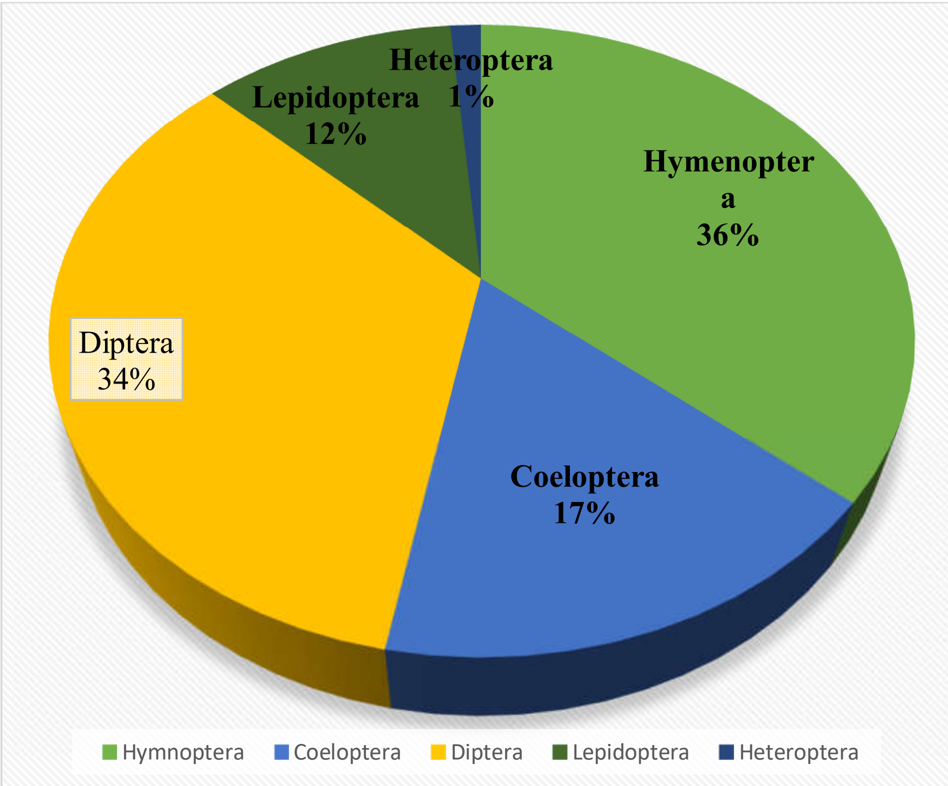


Figure 2. Order-wise composition of pollinator insects

The pollinator insects of mustard included five different orders. Among them, Hymenopterans (36%) was the most abundant order visiting mustard flowers followed by Dipterans (34%), Coelopterans (17%), Lepidopterans (12%) and Heteropterans (1%) was least abundant (Figure 2).

Table 1. Species diversity, their relative abundance and foraging sources

S.N.	Scientific name	Order	Family	Foraging source	Relative abundance (%)
1	*Apis cerana* Fabricius, 1793	Hymenoptera	Apidae	PN	24.35
2	*Apis mellifera* Linnaeus, 1758	Hymenoptera	Apidae	PN	11.28
3	*Bombus* sp.	Hymenoptera	Bombidae	PN	0.51
4	*Coccinella undecimpunctuta* (Linnaeus, 1758)	Coleoptera	Coccinellidae	C	7.69
5	*Coccinella septumpunctata* (Linnaeus,1758)	Coleoptera	Coccinellidae	C	9.07
6	*Episyrphus balteatus* De Geer, 1776	Diptera	Syrphidae	PN	12.51
7	*Eristalis* sp.	Diptera	Syrphidae	PN	19.33
8	*Musca domestica* (Linnaeus,1758)	Diptera	Muscidae	PN	2.41
9	*Aglais cashmiriensis* (Kollar,1848)	Lepidoptera	Nymphalidae	PN	1.64
10	*Pieris canidia* (Sparrman,1768)	Lepidoptera	Pieridae	N	1.84
11	*Vanessa cardui* (Linnaeus, 1758)	Lepidoptera	Nymphalidae	N	2
12	*Junonia lemonias* (Linnaeus, 1758)	Lepidoptera	Nymphalidae	N	1.69
13	*Lampides boeticus* (Linnaeus,1767)	Lepidoptera	Lycaenidae	N	1.23
14	*Neptis hylas* (Linnaeus, 1758)	Lepidoptera	Nymphalidae	C	1.33
15	*Eurema hecabe* (Linnaeus, 1758)	Lepidoptera	Pieridae	C	1.69
16	*Eurydema* sp.	Heteroptera.	Pentatomidae	C	1.38

Note: PN= Pollen and nectar, N=Nectar and C=Causal visitor.

Two different families of Hymenoptera were recorded among them Apidae was dominant family (35.64%) and Bombidae least abundant. Among Diptera, Syrphidae was most abundant family (31.84%) and Muscidae (2.41%). In Coeloptera, only one family

Cocinnelidae was present (16.67%), whereas Lepidoptera consists of three families- Nymphalidae (6.66%), Pieridae (3.53%) and Lycaenidae (1.23%). Heteroptera consists of family Pentatomidae (1.38%) of insect abundance (Figure 3).

Honeybees were the most abundant group of pollinators. Among honey bees, *Apis cerana* was the most dominant one (24.35%) and *Apis mellifera* (11.28%). Two species were found from family Syprihidae, among them *Eristalis* sp. was second most abundant (19.33%) and *Episyrphus balteatus* (12.51%) was third most abundant species visiting mustard flowers. Butterfly species like *Aglais cashmiriensis, Pieris canidia, Vanessa cardui, Junonia lemonias, Lampiides boeticus, Neptis hylas* and *Eurema hecabe* were also found visiting mustard flowers for nectar purposes but their abundance was very less compared to honey bees. Bumble bee the important pollinators of many agriculture crops, constitute only 0.51% of all the insect pollinators in mustard crops. Housefly (*Musca domestica)*, lady bird beetle like (*Coccinella undecimpunctuta* and *Coccinella septumpunctata)* and bug (*Eurydema* sp.*)* were also recorded from mustard field (Table 1).

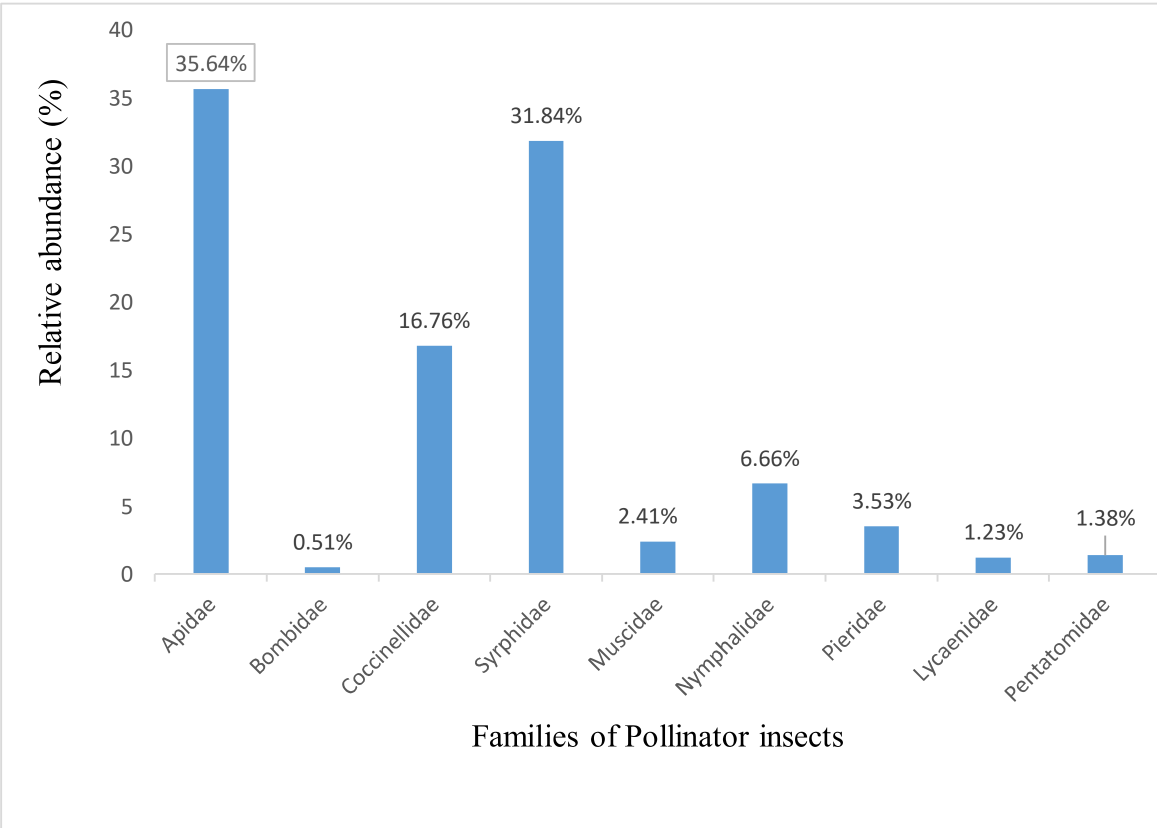


Figure 3. Families of Pollinator insects with their Relative abundance

4.4. Foraging sources of Pollinator insects

Among the recorded 16 species, seven species were found foraging on both pollen and nectar of mustard flowers. Of them, Hymenopteran species were the most common followed by Dipterans. Pollen and nectar feeding insects includes *Apis cerana, Apis melifera, Bombus* sp., *Eristalis* sp., *Episyrphus balteatus, Musca domestica* and *Aglais cashmiriensis.* Four species were found foraging on nectar it mostly includes butterfly species like *Pieris canidia, Vanessa cardui, Junonia lemonias* and *Lampiides boeticus.* The rest five species were recorded as causal visitor of the mustard flowers and it includes *Coccinella undecimpunctuta, Coccinella septumpunctata, Neptis hylas, Eurema hecabe* and *Eurydema* sp. (Figure 4).

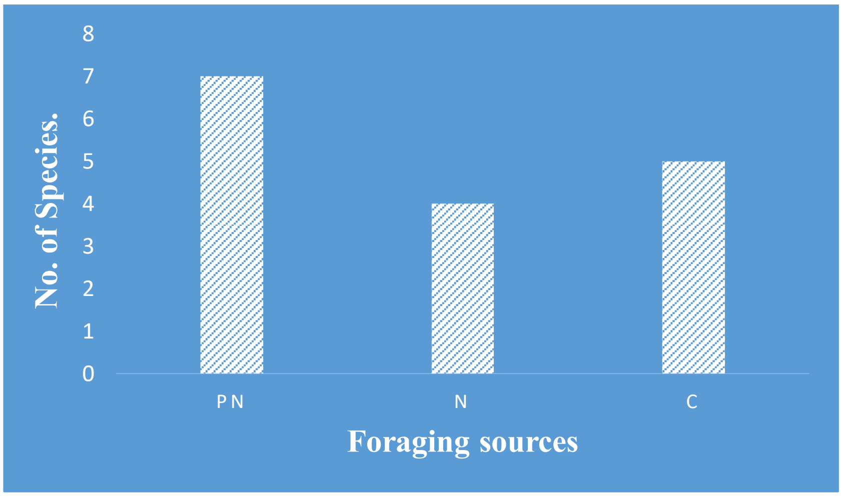


Figure 4. Foraging sources of insects

4.5 Foraging activities of insects

Although throughout the day all the insect groups were found active but their peak foraging activity time were different. The peak foraging activities of the members of Hymenoptera and Diptera were observed between 12 hr to 14 hr in which there were 12.88 individuals/1m^2/2 min and 13 individuals/1m^2/2min respectively. Butterflies were less active in the morning and their activities becomes peak at 14 hr comprising 2.55 butterflies/1m^2/2 min. Coelopterans foraging activities remain fairly constant throughout day, whereas Heteropterans foraging became peak at 14 hr to 16 hr (Figure 5).

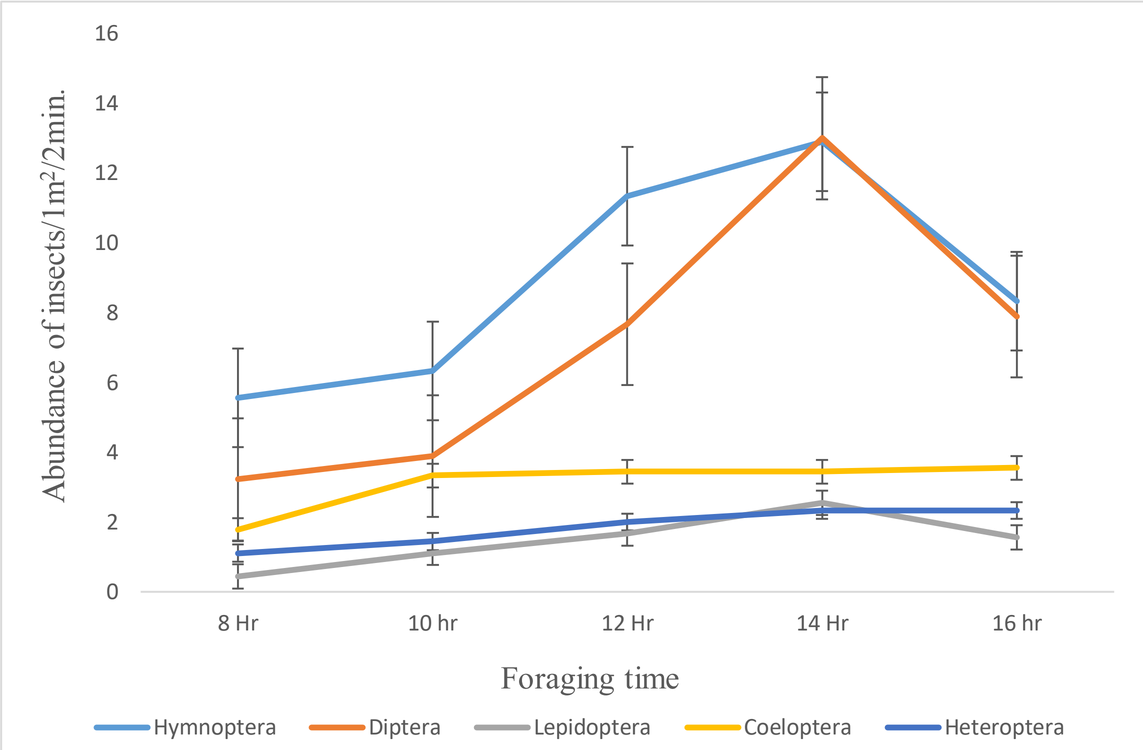


Figure 5. Foraging activities of different orders of pollinator insects

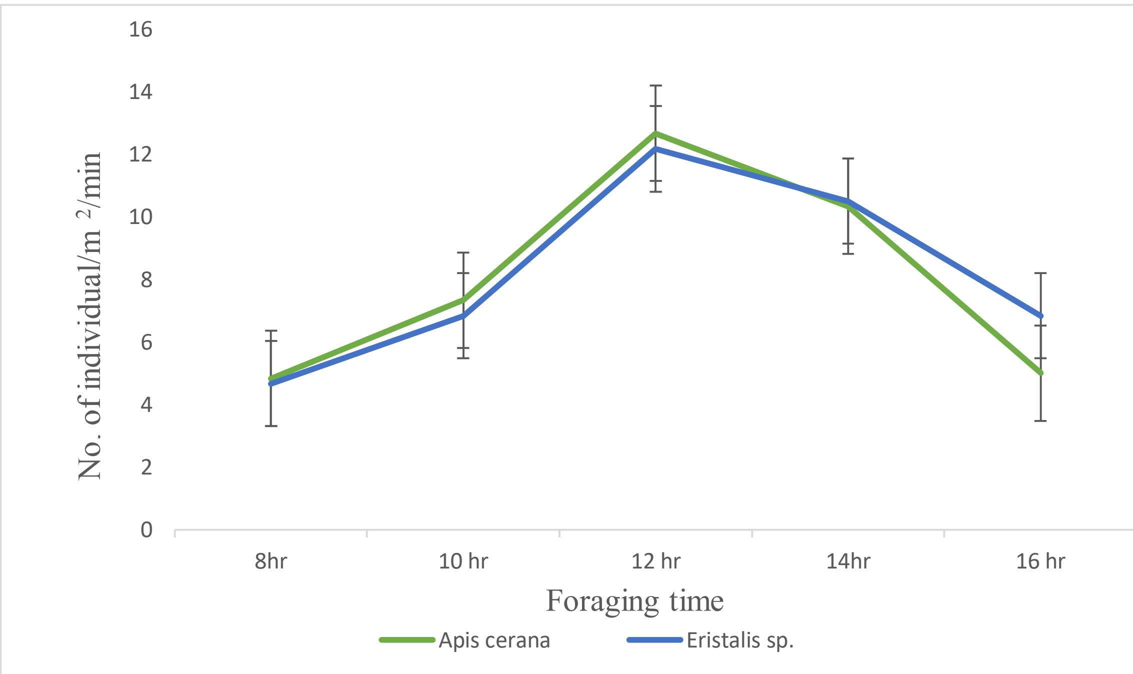


Figure 6. Foraging activities of *Apis cerena* and *Eristalis* sp.

Apis cerana and *Eristalis* sp. were the most abundant pollinator insect of mustard flower. They start their foraging activities from early morning and reaching peak activities at 12 hr. with 12.66 and 12.16 individuals/m^2/2 min. respectively and slowly declines as evening approach (Figure 6).

4.6 Relative abundance of pollinator insects during different phases of mustard blooming

Statistically, a significant difference in the abundance of pollinator insects was observed during different phases of flowering (Table 3). The highest abundance of insects were found during peak phase of flowering (11.74 individual/m^2/2min) followed by early phase (7.72 individual/m^2/2min. and least in late phase (5.96 individual/m^2/2min.) as an average of 25 observations (Table 2 and Figure 7).

Table 2. Mean no. of insects/m^2/2min in different phases of flowering

Phases	Mean	S.D
Early	7.72	1.10
Peak	11.24	2.36
Late	5.96	1.01

***average of 25 observations**

Table 3. Relation between pollinator insect's abundance with different phases of flowering

Dependent variable	Independent variable	Df	F	P
Abundance	Phases	2,72	18.844	**0.001**

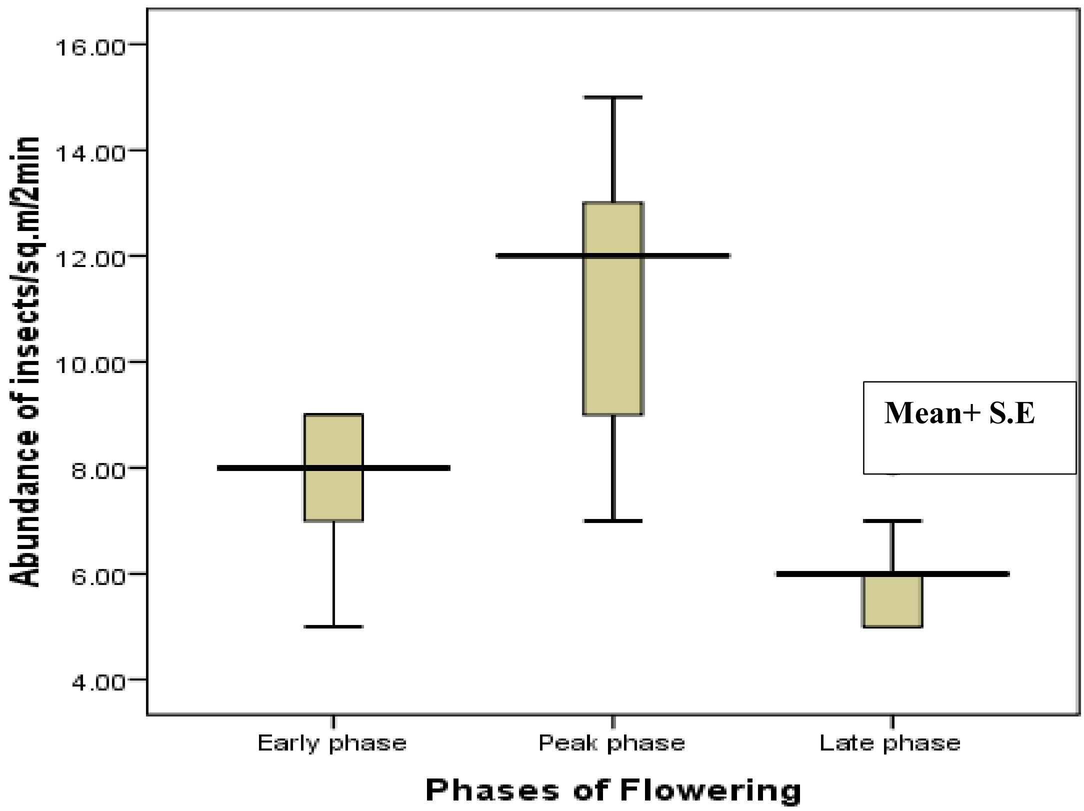


Figure 7. Abundance of pollinator insects during different phases of flowering

4.7. Effect of insect pollination on crop yield of mustard

Table 4 . Linear mixed effect models describing the effect of treatment on control and experiment plot obtained from ANOVA

Dependent variables	Independent variables	df	F	P
Height of plant (cm)	Treatment	1,78	0.224	0.638
No. of branches	Treatment	1,78	15.308	**0.001**
No. of flowers	Treatment	1,78	0.316	0.576
No. of pods	Treatment	1,78	11.651	**0.001**
Fruit set %	Treatment	1,78	47.687	**0.0001**
Length of pod (cm)	Treatment	1,78	1.981	0.163
No. of seed per pod	Treatment	1,78	39.526	**0.0001**
Diameter of seed(mm)	Treatment	1,78	498.339	**0.002**

Significant codes: 0.000= highly significant, 0.001= moderately significant, 0.01= significant, 0.05= marginal significant.

4.7.1 Effect on height of plant

The effect of insect pollination on height of plant was not significant difference as $p>0.05$ (Table 4). The mean height of mustard plant on experimental and control plot were 54.20 ± 1.458 and 53.12±1.811cm respectively (Table 5 and Figure 8. a).

4.7.2 Effect on number of branches

The effect of insect pollination on number of branches in a plant was statistically significant difference as $p<0.05$ (Table 4).The mean of number of branches in a plant on experimental and control plot were 5.025 ± 0.18 and 4.05 ± 0.17 respectively (Table 5 and Figure 8.b)

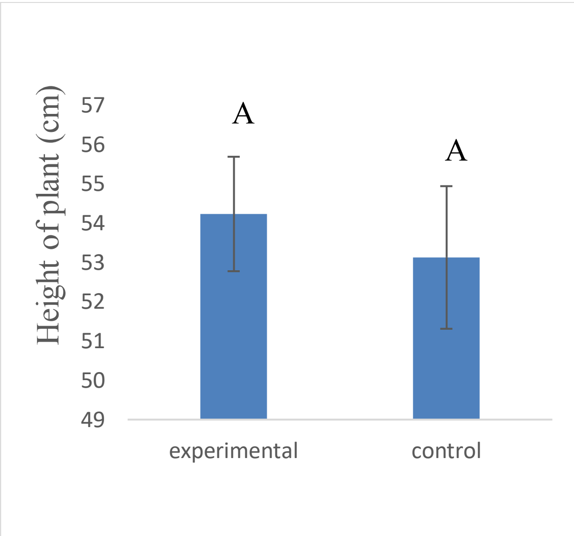


Figure 8 (a). Effect on height plant

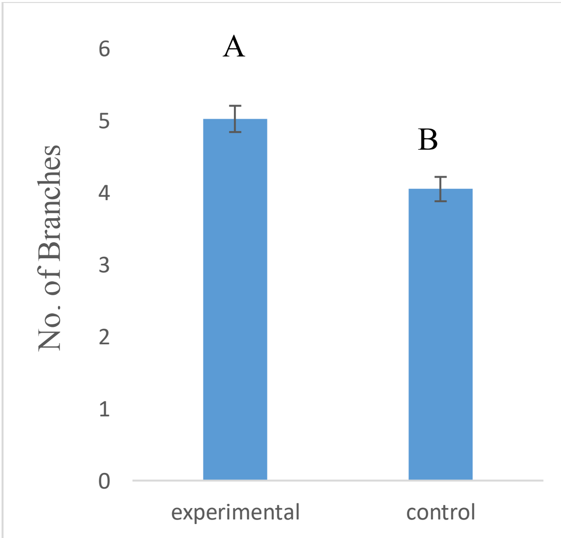


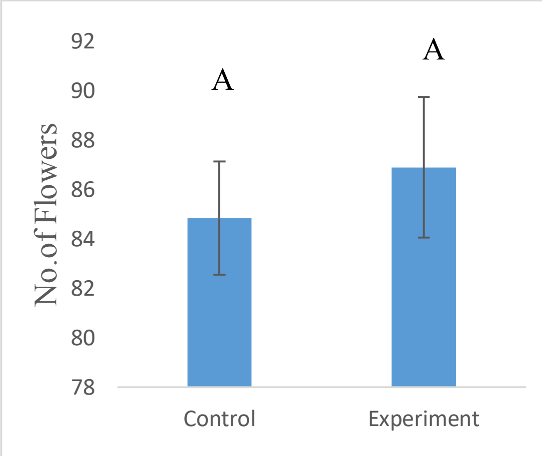
Figure 8 (b). Effect on number of branches

4.7.3 Effect on number of flowers

The effect of insect pollination on number of flowers in a plant was not significant difference as $p>0.05$ (Table 4).The mean number of flowers on experimental and control plot were 86.90±2.848 and 584.85±2.281cm respectively (Table 5 and Figure 8.c).

4.7.4 Effect on number of pods

The effect of insect pollination on number of pods in a plant was significant difference as $p<0.05$ (Table 4). The mean of number of pods in a plant on experimental and control plot were 70.47 ±2.43 and 59.80 ±1.96 respectively (Table 5 and Figure 8.d).



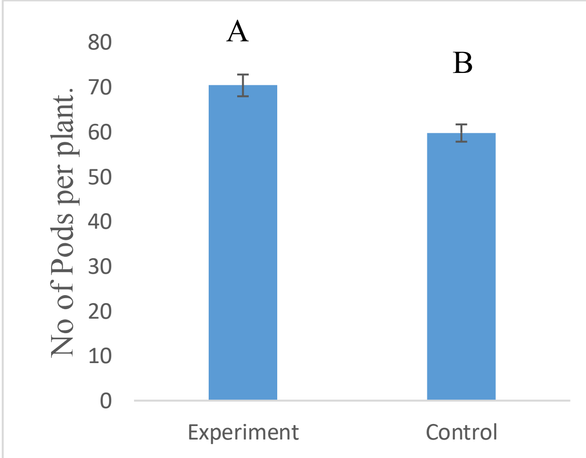


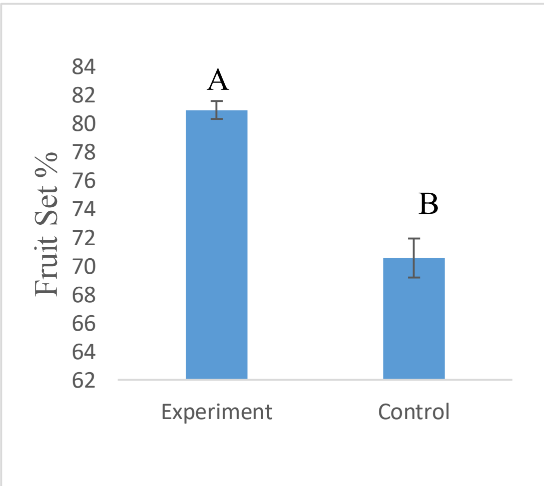
Figure 8(c). Effect on number of Flowers **Figure 8(d). Effect on number of pods**

4.7.5 Effect on Fruit set (%)

The impact of pollination by insects significantly increases the percentage of fruit set resulting in higher number of pods. There was significant difference between the fruit set percentage between experimental and control plot as $p<0.05$ (Table 4). The mean percentage of fruit set on experimental and control plot were 80.94 ±0.638 and 70.55 ± 1.362 % respectively (Table 5 and Figure 8.e).

4.7.6 Effect on length of pod

There was no impact of insect pollination on length of pod as there was no significant difference in the length of pod between experimental and control plot as $p= 0.163$ (Table 4). The mean length of pod on experimental and control plot were 5.80 ±0.135 and 5.50 ± 0.164 cm respectively (Table 5 and Figure 8.f).



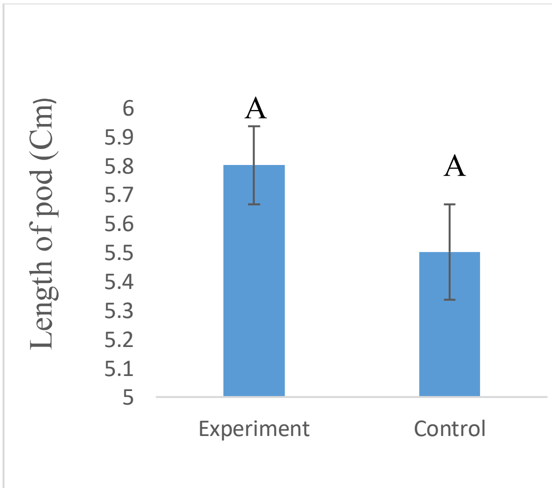


Figure 8(e). Effect on Fruit set % **Figure 8(f). Effect on Length of pod**

4.7.7 Effect on number of seeds per pod

The impact of pollination by insects significantly increases the number of seeds per pod. There was significant difference in the number of seeds per pod between experimental and control plot as $p<0.05$ (Table 4). The mean number of seed per pod on experimental and control plot were 19.30 ±0.33 and 16.70 ± 0.248 respectively (Table 5 and Figure 8.g).

4.7.8 Effect on diameter of seed

The effect of insect pollination on diameter of seed was statistically significant difference as $p<0.05$ (Table 4). The mean diameter of seed on experimental and control plot were 0.279 ±0.051 and 0.133±0.040mm respectively (Table 5 and Figure 8.h).

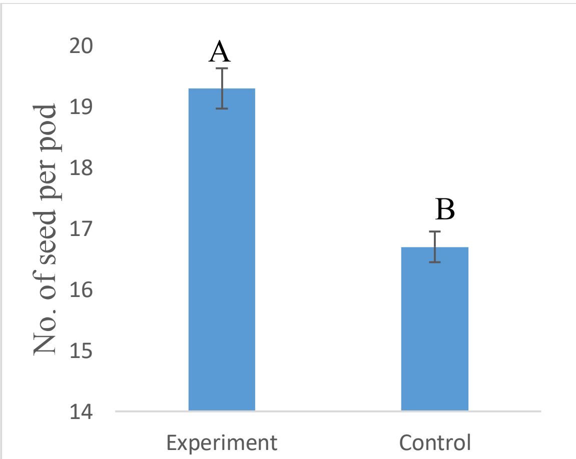


Figure 8(g). Effect on number of seeds per pod

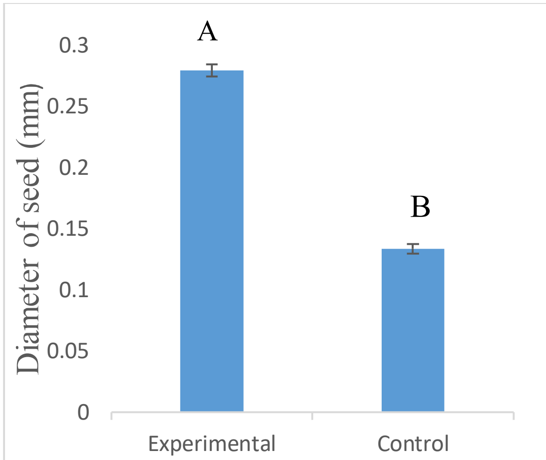


Figure 8(h). Effect on diameter of seed

4.7.9 Effect on mean weight of 100 dry seeds

There was statistically significant difference in weight of 100 dry seeds between experimental and control plot. The mean weight of 100 dry seeds on experimental and control plot were 0.48±0.023 and 0.33±0.058 gm. respectively (Table 5 and Figure 8.i).

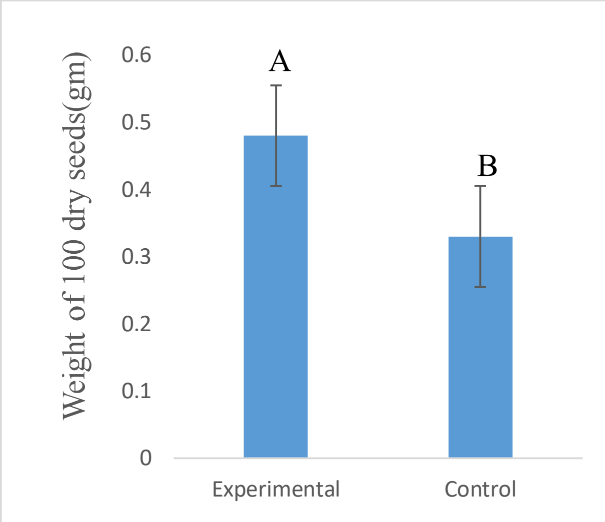


Figure 8 (i). Effect on weight of 100 dry seeds

Note: Bars with same letter are not significantly different at the 5% significance level

Table 5. Effects of open pollination (experiment) and caging of mustard plants (control) on some qualitative and quantitative parameters

S.N.	Parameter	Control *	Experiment*	% Increase
1	Height of plant(cm)	53.12±1.811 'a'	54.22±1.459 a	2.07
2	No. of branches	4.050±0.171 a	5.025±0.180 b	24.07
3.	No of flowers	84.85±2.281 a	86.90±2.848 a	2.41
4	No. of pods	59.80±1.967 a	70.47±2.431 b	17.84
5	Fruit set (%)	70.55±1.362 a	80.94±0.638 b	14.72
6	Length of pods (cm)	5.50±0.164 a	5.802±0.135 a	5.490
7	No of seeds per pods	16.70±0.248 a	19.30±0.330 b	15.56
8	Diameter of seeds(mm)	0.133±0.2547 a	0.275±0.0051 b	106.76
9	Weight of 100 dry seeds (gm)	0.33±0.058 a	0.48±0.023 b	45.45
10	Colour and shape of seeds	Light brown & shrink	Deep brown & round	-------

Note: *= Mean± S.E and Means marked with same letter (row wise) are not Significantly different (p>0.05)

4.7 Relationship between length of pod and number of seeds

There was positive correlation between length of pod and number of seeds. The Pearson correlation matrix showed that number of seeds per pod increases with the increase in length of pod and relationship is significantly strong in experimental plot than the control plot (Table 5).

Table 6. Correlation between length of pod and number of seeds

Pearson Correlation Matrix (r)	Control	Experiment
	0.563**	0.652**

**** Correlation is significant at 0.01 level (2 –tailed)**

5. DISCUSSION

5.1 Pollinator insect diversity and their relative abundance

The pollinator insects of mustard included 16 species under five different orders. Among them, Hymenopteran (36%) was the most abundant insect order visiting mustard flowers followed by Diptera (34%), Coleoptera (17%), Lepidoptera (12%) and the lowest Heteroptera (1%). Similar results on insect's visitors were reported by Dhakal (2003) on rapeseed field. Devi *et al*. (2017) also recorded a total of 88 insects belonging to 63 genera under 31 families and nine orders were found to visit mustard flowers, Hymenoptera was the most abundant insect order. The results of the present investigation almost similar to those reported by early workers. Kunjwal *et al*. (2014) observed a total of 30 species belonging to four orders Hymenoptera, Diptera, Lepidoptera, and Coeloptera visiting mustard, *Brassica juncea* flowers. Among them Hymenoptera were the major insect pollinators. Similarly Kamel *et al*. (2015) observed 21 species of insect pollinators visiting canola, *B. napus* flowers. Bhowmik e*t al*. (2014) recorded 19 different insect species under four orders. Among them, order Lepidoptera and Diptera shared maximum number of species followed by Hymenoptera and Coleoptera, of them, Hymenopteran species were reported to be common with significantly active throughout the day, followed by Coeloptera, Diptera and Lepidoptera. In this study, Order Coleoptera was also reported to be quite high (17%) and it was probably because of their predatory action on the aphids that are commonly found on mustard flower. Nine families of insect pollinators were recorded and among them family Apidae was the most abundant, followed by Syrphidae. These findings are similar to those recorded by Pudasaini *et al*. (2015) where they recorded seven families of Hymenoptera and Apidae was dominant family. Goswami and Khan (2014) also recorded six familes of Hymenoptera and Apidae being most abundant, Syrphidae was the most dominant among the true flies (Ali *et al*., 2011). Bombidae was present in less abundance as there was not favourable condition for them as many wild flowers were absent as study was done in peak winter.

Among the recorded 16 species most of them also appear as major pollinator insects of mustard. These includes honeybees, flies and butterflies. The yellow colour of the flower with shallow placement of visible nectar mostly attracts bees, flies and butterflies (Ali *et al*., 2011). *Apis cerana* was the most abundant pollinator insects in this study which is similar to the findings of Mishra *et al*. (1988), but Pudasaini *et al.* (2015) and Kunjwal *et al.* (2014) argued that among many insect flower visitors *Apis mellifera* was the most common pollinating species of mustard. But higher abundance of *Apis cerana* in this study was due to higher number of *Apis cerana* colonies rearing by farmers near the study area. Similarly, Shakeel and Inayatullah (2013) recorded nine species of pollinator insects in canola, among which *A.mellifera, A. florea, A. dorsata,* and *A. cerana* were the major pollinators those findings were similar to present present study. Butterflies species like *Aglais cashmiriensis, Pieris canidia, Vanessa cardui, Junonia lemonias, Lampiides boeticus, Neptis hylas* and *Eurema hecabe* were present in less abundance. These findings are similar to those reported by early workers Bhowmik *et al*. (2014) and Pudasaini *et al*. (2015). Butterflies were more limited floral visitors both in abundance and diversity (Ali *et al*., 2015). Hoverflies species like *Eristalis* sp. and *Episyrphus balteatus* were also present in relatively high abundance. Several hoverflies species, although linked to forest landscape are dependent on access to multiple alternative resources to complete their life cycles. They, therefore benefitted from a high proportion of agricultural habitats in the surrounding landscape possibility due to availability of larval food and pollen and nectar for adult (Kleijn *et al*., 2004). These may be one of the reasons for high abundance of hoverflies. Suprisingly, abundance percentage of *Coccinella undecimpunctuta* and *Coccinella septumpunctata* was reported to be quite high and it was probably because of their predatory action on the aphids that are commonly found on mustard.

5.2 Foraging sources and activities of pollinator insects during mustard flowering

Mustard field harbored many species of insects that collected nectar and pollen. Among the recorded 16 species, seven species were found foraging on both pollen and nectar of mustard flowers. Of them Hymenopteran species were the most common followed by Dipterans. The open field attracted bees in higher number, which were beneficial for both nectar and pollen collectors (Rijal *et al*., 2017). Four species were found foraging on nectar it mostly includes butterfly species and the rest five species were recorded as causal visitor of the mustard flowers. The present findings are in line with Soliman *et al*. (2015) who reported that all Hymenoptera visitors were observed as both pollen and nectar foragers, whereas all Diptera and Lepidoptera species were observed as nectar foragers and only accidently transferred pollen, and species of Coleoptera were causal visitors of canola flowers and were not participating in nectar or pollen foraging. Similarly, Roy *et al*. (2014) found that among six species of hymenopterans, four species were found as pollen and nectar foragers and two species as only nectar foragers. All Dipterans and Lepidopterans were found as only nectar foragers and others like Coleoptera, Odonata and Hemiptera species as causal visitors of mustard flowers.

Although, throughout the day all the insect groups were found active but their peak foraging activity time were different. The peak foraging activities of the members of Hymenoptera and Diptera were observed between 12hr to 14 hr in which there were 12.88 individuals/1m^2/2 min and 13 indviduals/1m^2/2min respectively. Butterflies species were less active in the morning and their activities becomes peak at 14 hr comprising 2.55 butterflies/1m^2/2 min. Coelopteran species foraging activites remain fairly constant throughout day, whereas Heteropteran species foraging became peak at 14 hr to 16 hr. These findings are similar to Bhowmik *et al*. (2014) who found that the peak foraging activity of the members of Hymenoptera, Coleoptera, Lepidoptera and Diptera was observed to be at 12 p.m., 1 p.m., 12 p.m. and 2 p.m. respectively. But, Roy *et al*. (2014) argued the peak foraging activity of the members of Hymenoptera, Coleoptera, Diptera and Hemiptera at 2 p.m and that of Lepidopterans were found during 12 p.m. Maximum honey bee activity was recorded in the 12-14 hr as it provides more floral rewards in terms of pollen which is regarded as source of protein. A diurnal rhythm of honey bee visits is by change in climatic conditions. Bee visits commenced only if the threshold level of temperature and light intensity is surpassed (Sihag, 1984). During afternoon, especially on winter days bees receive optimum temperature and light intensity to increase their foraging activity. This was reason for peak foraging of bees between 12-14 hr. The maximum abundance of all insect pollinators at morning hours is due to variation in atmospheric condition and availability of floral rewards from large number of freshly opens flowers (Priti and Sihag 1998; Sihag and Khatkar, 1999). Free (1993) stated that the metabolic activity of insects increases as the temperature increases and they visit many flowers at that time. Honeybee is homeo-thermal in nature, as the colony can regulate their body temperature (Sihag, 1984). However, an individual honeybee is poikilothermic and it cannot regulate its body temperature (Michener, 1974)**.** This may be the reason that *Apis cerana* foraging becomes peak at 12 hr. *Apis cerena* and *Eristalis* sp. were the most abundant pollinator insect of mustard flower. They start their foraging activities from early morning and reaching peak activities at 12 hr. with 12.66 and 12.16 individuals/m^2/2 min. respectively and slowly declines as evening approach. Bee activity was noticed to decline as the day advanced (Soliman *et al*., 2015)

There was statistically significant difference in the abundance of pollinator insects during different phases of flowering. The highest abundance of insects were found during peak phase of flowering followed by early phase and least in last phase. This may due to the fact that at peak flowering stage there are large number of flowers that attract insects for pollen and nectar. Honey bee density in mustard plant were positively correlated with flower density (Abrol, 2007).

5.3 Impact of pollination on crop yield of mustard

The effect of insect pollination on height of plant was not significant difference. This findings are in line with Aryal *et al*. (2014) who reported there was no significance difference on the plant height of buckwheat up to 45 DAS, i.e. before flowering whereas Rijal *et al*. (2017) concluded that plant height was significantly lower in pollination treatments than in the control treatment. The non-significance on the plant height might be due to homogeneity in field and no treatment difference among the plots, except insect pollination.

The effect of insect pollination on number of branches in a plant was statistically significant difference, similar results was interpreted by Rijal *et al.*(2017) in which there was significant difference in the number of branches in open and close treatment. The increases in branches may be due to insect herbivory. Plants had significantly higher number of branches and leaves in presence of grass species (Scherber *et al*., 2006).

The effect of insect pollination on number of flowers in a plant was not significant difference, it may be due to homogeneity in field and no treatment difference among the plants. Flowering is a natural process and pollution had no influence on it.

The effect of insect pollination on number of pods in a plant was statistically significant difference as it increases pod number upto 17.84% between control and experimental plot. The present findings are in line with the findings of Kumari *et al*. (2013) who reported that the maximum number of pods per plant in *Brassica juncea* was observed in open pollinated plots which were significantly higher than that in *A*. *mellifera* pollinated plots and significantly the lowest were observed in pollinators' exclusion. Thakur and Karnatk (2005) reported that highest number of pods per plant in insect pollinated plants then caged plants without pollinators. Free and Nutall (1968) observed that *B. juncea* plants caged with bees produced 25 per cent more seed than plant caged without bees. Parsad *et al*. (1989) found highest yield of *B. juncea* in open pollinated plot, whereas caged plots (excluding pollinators) yield the lowest.

There was significant difference between the fruit set percentage between experimental and control plot having 80.94 % and 70.55% respectively with the increase of 14.72% fruit set between control and experimental plot. This study suggests that insect pollinators are playing an important role in seed set of mustard crop. The results of present investigation are in conformity with the earlier recorded observations of Tara and Sharma (2010) on *Brassica campestris* var. *sarson*, which revealed that seed set, was less (79.96%) in controlled experiment as compared to open pollinated flowers (88.05%). Goswami and Khan (2014) also studied the impact of different modes of pollination in Mustard (*Brassica juncea* L.: Cruciferae) and reported that highest percent pod set was in open pollinated (83.42%) plots followed by bee pollinated (75.41%) and caged pollinated (62.80%) and recorded an increase of 8.09% pod set in open pollinated flowers as compared to controlled ones. Similar observations were also reported by Singh (1997) on *Brassica juncea* and Singh *et al.* (2004) on var. *toria*.

There was no significance differences in length of pod between experimental and control plot. It was also determined that length pod increases by 5.4% but insect pollination have nothing relation with it. There was significant difference in number of seeds per pod, diameter of seeds and weight of 100 dry seeds between experimental and control plot. These findings are similar to Devi *et al*. (2017) who revealed that the seed per siliqua and 1000 seed weight were significantly higher in open pollination (15.49 and 15.59 seed per siliqua) followed by hand pollination (14.25 and 14.18 seed per siliqua) during 2015 and 2016, respectively. Significantly less seed 12.16 and 12.14 seed per siliqua was recorded in pollinators' exclusion over the two years of study The mean thousand weight of mustard seed was significantly more in open modes of pollination (3.11 and 3.12g) followed by that in hand pollination (2.95 and 2.98g) during 2015 and 2016. The lowest mean thousand seed weight (2.36g) was recorded in pollinator's exclusion over the two year of study. The results of present investigation corroborate the observations made by Singh and Singh (1992) who reported that insect pollinated plots produced three times heavier seed then self-pollinated plants in *B. campestris* var. *toria*. The present findings are also corroborated by the results of Kamel *et al.* (2015) who observed that the weight of 1000 seeds was higher in open pollinated plants (3.13 g) than those of caged plants (2.4 g) in *B. napus*.

A positive correlation between length of pod and number of seeds was obtained. The Pearson correlation matrix showed that number of seeds per pod increases with the increase in length of pod and relationship is significantly strong in experimental plot than the control plot. This findings are similar to Ali *et al*. (2011) who recorded that seeds weight per pod increased with increasing number of seeds per pods.

6. CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Mustard flowers were visited by 16 pollinator insect species belonging to five insect orders (Hymenoptera, Diptera, Lepidoptera, Coleoptera and Heteroptera) belonging to nine families (Apidae, Bombidae, Syrphidae, Muscidae, Nymphalidae, Pieridae, Lycaenidae, Coccinellidae and Pentatomidae). Hymenoptera was the most abundant order followed by Diptera whereas Heteroptera was least abundant. Apidae was most abundant family followed by Syrphidae. *Apis cerana* was the most dominant species and *Eristalis* sp. was second most abundant in mustard flower.

Among the recorded 16 species, seven species were found foraging on both pollen and nectar, four species foraging only nectar of mustard flowers and remaining five as causal visitors. The peak foraging activities of the members of Hymenoptera and Diptera were observed between 12hr to 14 hr. Butterflies were less active in the morning and their activities becomes peak at 14 hr, Coelopterans foraging activities remain fairly constant throughout day, whereas Heteropteran species foraging became peak from 14 hr to 16 hr. There was significant difference in the abundance of pollinator insects during different phases of flowering.

Statistically, a significant difference was observed in number of branches, number of pods, fruit set, number of seeds per pods, diameter of seed and weight of 100 dry seeds whereas, the difference was non- significant in case of height of plant, number of flowers and length of pod. There was positive correlation between length of pod and number of seeds.

In conclusion, it may be stated that decline in the species diversity could pose a serious threat on crop plant pollination and seed production. Though it was a preliminary attempt to make a report of insect pollinators of mustard crops from Kusma, Parbat, it will certainly help the future workers as a baseline data of pollinators and pollination crops in the area. Hence, pollinator's friendly cultivation practices should be practiced for conservation and management of insect pollinators for higher production and productivity of mustard.

6.2 Recommendations

Pollinators plays an important functional role in most terrestrial ecosystem and provide a key ecosystem service. Insects, particularly bees, are the primary pollinators for the majority of the world's angiosperms. Without this service, many interconnected species and processes functioning within both wild and agricultural ecosystem could collapse. Some of the recommendation are

1. It is needed to evaluate the contribution of insect pollination to seed or fruit set for other widely cultivated crop species too.

2. To estimate the contribution to the quality and market value of mustard (oil content, germination percentage and chlorophyll contents).
3. Awareness program regarding the importance and conservation of pollinator insects should be carried out from farmer's level.

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APPENDICES

APPENDIX 1. Shannon-Wiener diversity indices (H) and eveness index (J) of pollinator insects

S.N	Species	Ni	Pi	Ln Pi	Pi*LnPi
1	*Apis cerana*	475	0.24359	-1.41227	-0.34401
2	*Apis melifera*	220	0.112821	-2.18196	-0.24617
3	*Bombus* sp.	10	0.005128	-5.273	-0.02704
4	*Coccinella undecimpunctuta*	150	0.076923	-2.56495	-0.1973
5	*Coccinella septumpunctata*	177	0.090769	-2.39943	-0.21779
6	*Episyrphus balteatus*	244	0.125128	-2.07842	-0.26007
7	*Eristalis* sp.	377	0.193333	-1.64334	-0.31771
8	*Aglais cashmiriensis*	32	0.01641	-4.10985	-0.06744
9	*Pieris canidia*	36	0.018462	-3.99207	-0.0737
10	*Vanessa cardui*	39	0.02	-3.91202	-0.07824
11	*Junonia lemonias*	33	0.016923	-4.07908	-0.06903
12	*Lampides bacticus*	24	0.012308	-4.39753	-0.05412
13	*Neptis hylas*	26	0.013333	-4.31749	-0.05757
14	*Eurema hecabe*	33	0.016923	-4.07908	-0.06903
15	*Musca domestica*	47	0.024103	-3.72544	-0.08979
16	*Eurydema* sp.	27	0.013846	-4.27975	-0.05926

Shannon-Weiner diversity index (H)	2.2283
H_{max}	2.772589
Eveness index (J)	0.803689

APPENDIX 2. Analysis of Qualitative and quantitative parameters of mustard plant for control plot

Plant no.	Height of plant (cm)	No. of flowers	No of pods	Fruit set (%)	No of Branches	Length of pod (cm)	No. of Seeds	Diameter of seeds (mm)
CA1	82	83	45	54.21	6	7	20	0.12
CA2	65	92	74	80.43	5	5.6	15	0.15
CA3	38	50	34	68	4	5.7	16	0.1
CA4	45	74	45	60.81	4	6	18	0.19
CA5	63	110	75	68.18	4	6.3	16	0.13
CA6	42	95	53	55.78	4	6.6	17	0.13
CA7	45	86	62	72.09	3	7	18	0.12
CA8	54	76	55	72.36	4	5	15	0.13
CA9	57	83	51	61.44	5	4.6	15	0.14
CA10	61	67	42	62.68	3	4.8	16	0.16
CB1	38	78	58	74.35	4	5	17	0.14
CB2	43	75	61	81.33	6	5.4	17	0.13
CB3	46	98	71	72.44	4	5	16	0.14
CB4	56	106	82	77.35	3	6	17	0.12
CB5	73	118	83	70.33	3	5	16	0.1
CB6	47	89	54	60.67	3	6.2	17	0.1
CB7	54	79	45	56.96	2	3.5	15	0.11
CB8	58	85	50	58.82	5	6	16	0.12
CB9	71	84	62	73.80	2	4.5	15	0.16
CB10	39	75	55	73.33	3	4	18	0.15
CC1	44	72	58	80.55	5	3.5	15	0.11
CC2	42	84	59	70.23	4	4	16	0.16
CC3	63	89	67	75.28	5	5	16	0.16
CC4	37	96	72	75	5	5.6	17	0.15
CC5	43	98	68	69.38	3	6.3	19	0.2
CC6	50	104	76	73.07	5	6.7	21	0.17
CC7	63	105	81	77.14	6	6.2	20	0.1
CC8	38	98	72	73.46	3	5.6	17	0.16
CC9	73	90	69	76.66	4	4.8	16	0.14
CC10	65	87	59	67.81	3	4.7	15	0.12
CD1	56	88	63	71.59	5	7	19	0.14
CD2	44	76	52	68.42	6	7.2	18	0.14
CD3	56	85	48	56.47	5	6.8	17	0.12
CD4	58	65	49	75.38	5	5.8	15	0.11
CD5	52	75	58	77.33	4	6.5	16	0.1
CD6	60	63	54	85.71	3	3.7	14	0.17

CD7	48	56	48	85.71	4	6.5	17	0.12
CD8	55	96	83	86.45	3	4	16	0.1
CD9	37	75	46	61.33	4	5	16	0.11
CD10	64	89	53	59.55	3	6	18	0.12

APPENDIX 3. Analysis of Qualitative and quantitative parameters of mustard plant for Experimental plot

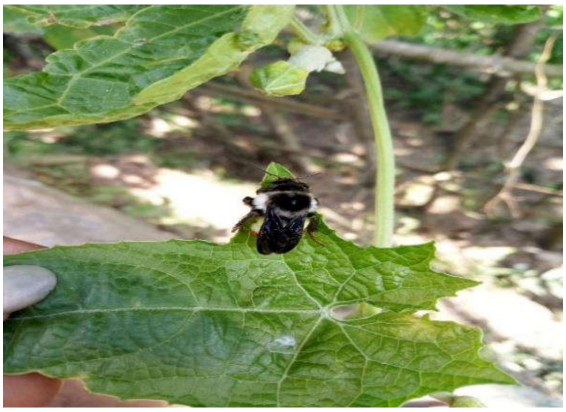
Plant No.	Height of plant(cm)	No. of Flowers	No. of pods.	Fruit set (%)	No. of branches	Length of pod(cm)	No. of seeds	Diameter of seeds (mm)
EA1	45	62	51	82.25	3	6	16	0.29
EA2	60	110	93	84.54	6	7	20	0.26
EA3	44	72	58	80.55	4	6	20	0.22
EA4	47	66	55	83.33	2	4.5	19	0.24
EA5	31	46	32	69.56	5	6	21	0.17
EA6	47	68	56	82.35	4	5	22	0.19
EA7	52	73	58	79.45	5	4	16	0.3
EA8	65	113	90	79.64	4	4	17	0.28
EA9	56	107	92	85.98	6	5.5	18	0.28
EA10	56	107	86	80.37	6	6	21	0.27
EB1	65	117	92	78.63	7	7	20	0.3
EB2	49	46	37	80.43	3	6.5	19	0.32
EB3	65	93	74	79.56	5	6	18	0.3
EB4	43	65	54	83.07	5	5.8	17	0.28
EB5	46	66	50	75.75	4	6	21	0.29
EB6	51	64	53	82.81	5	5.5	20	0.28
EB7	60	74	63	85.13	4	4.8	17	0.28
EB8	48	87	74	85.05	7	5	18	0.3
EB9	65	89	71	79.77	6	5.3	19	0.29
EB10	58	103	81	78.64	5	5.7	21	0.29
EC1	43	110	94	85.45	4	7	24	0.3
EC2	65	87	74	85.05	5	6.6	23	0.3
EC3	72	83	69	83.13	6	5.5	21	0.3
EC4	64	94	85	90.42	4	6	20	0.32
EC5	57	97	79	81.44	6	4.5	17	0.34
EC6	59	118	94	79.66	4	4.8	18	0.23
EC7	44	75	58	77.33	5	5.2	18	0.26
EC8	49	84	64	76.19	6	5.5	17	0.29
EC9	57	92	78	84.78	7	6	19	0.28
EC10	39	96	75	78.12	6	6.7	21	0.26
ED1	45	88	65	73.86	5	7.2	20	0.26
ED2	67	92	73	79.34	4	7	23	0.3
ED3	65	96	79	82.29	5	5.8	20	0.29
ED4	60	102	84	82.35	6	5.9	22	0.28
ED5	58	87	73	83.90	6	6.4	18	0.29
ED6	54	79	68	86.07	5	6.6	19	0.27

ED7	46	84	64	76.19	4	7	19	0.3
ED8	49	87	64	73.56	6	4.5	15	0.29
ED9	62	94	78	82.97	5	6	17	0.28
ED10	60	103	81	78.64	6	6.3	21	0.29

PLATE 1. Pollinator insects recorded from mustard field

Apis cerana

Apis mellifera

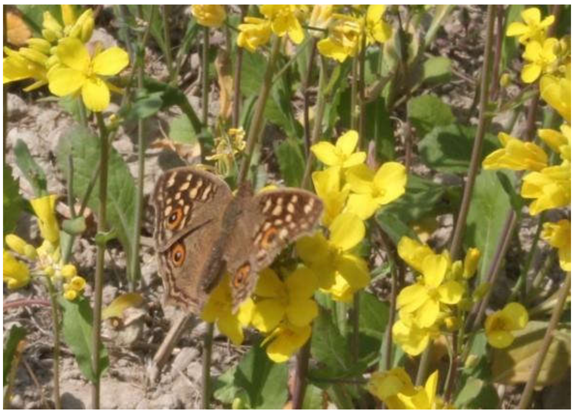


Bombus sp.

Episyrphus balteatus

Eristalis sp.

Vanessa cardui



Junonia lemonias

Coccinella septumpunctata

Coccinella undecimpunctuta

Neptis hylas

Aglais cashmiriensis

Pieris canidia

Eurydema sp.

Lampides bacticus

Eurema hecabe

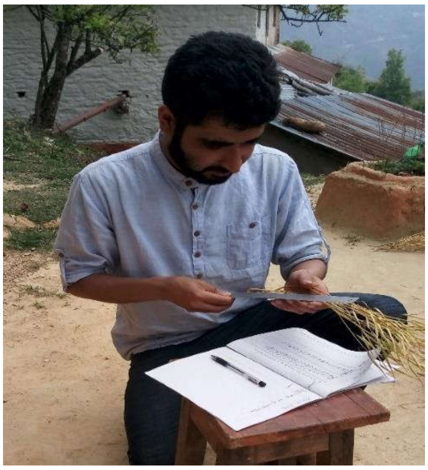
Musca domestica

PLATE 2. Researcher during field and lab work


Tagging of plants | Control plot | Yellow pan trap in operation



Experimental plot | Researcher collecting and preserving insects



Researcher analyzing various parameters during crop harvest