INSECT POLLINATOR AND WEED ECOLOGY IN A MANGO AGROECOSYSTEM

NURUL HUDA BINTI ABDUL

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INSECT POLLINATOR AND WEED ECOLOGY IN A MANGO AGROECOSYSTEM

By

NURUL HUDA BINTI ABDUL

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LIST OF SYMBOLS AND ABBREVIATIONS

CCA	Canonical Correspondence Analaysis
cm	Centimeter
CO_2	Carbon dioxide
CRD	Complete Randomized Design
df	Degree of freedom
^{o}C	Degree Celsius
ETOH	Ethanol
FAO	Food and Agricultural Organization
g	Gram
g/L	Gram per liter
L	Liter
ml	Milliliter
µm	micrometer
mm	Millimeter
m^2	Meter square
ms^{-1}	Meter per second
NPKMgO	Nitrogen, Phosphorous, Potassium, Magnesium oxide
RH	Relative Humidity
SE	Standard error
SDR	Species Diversity and Richness
UiTM	Universiti Teknologi MARA

EKOLOGI SERANGGA PENDEBUNGA DAN RUMPAI DI AGROEKOSISTEM MANGGA

ABSTRAK

Kepentingan pendebunga liar dalam pengeluaran buah mangga telah diketahui secara meluas tetapi taburan mereka di kawasan tropika dan hubungannya dengan tanaman masih kurang penghuraiannya. Untuk memahami peranan pendebunga liar dalam pengeluaran mangga, kajian ini menyiasat taburan serangga pelawat bunga (antofil) di jambak bunga dua kultivar mangga, *Mangifera indica* L. kv. ‘Sala’ dan ‘Chok Anan’. Kelimpahan antofil telah dipantau melalui 15 minit pungutan dalam setiap jam dari 0800 am sehingga 1500 pm, pada selang 4 hari bermula dari awal berbunga sehingga semua bunga telah kering (12-28 Februari 2013 dan 28 Januari 2014 - 7 Mac 2014). Antofil dari 10 order serangga yang terdiri daripada 79 famili, 156 genus dan 15803 serangga telah melawat bunga mangga. Hymenoptera (38.54 %) adalah order yang paling dominan diikuti oleh Hemiptera (34.59 %) dan Thysanoptera (10.32 %). Chok Anan menarik jauh lebih banyak antofil berbanding Sala pada $P < 0.05$, $df = 264$, t = -7,490. Kelimpahan antofil berbeza mengikut keterdapatan bunga pada masa persampelan yang berbeza. Antofil terbang lebih banyak (min kelimpahan 34.98 ± 2.004) melawat bunga terutama semasa bunga mula kembang berbanding antofil merayap (24.44 ± 1.973). Herbisid glufosinat-amonium yang digunakan untuk mengawal rumpai di dapati tidak toksik kepada antofil dan kelimpahan mereka pada panikel Chok Anan dan Sala tidak terjejas ($P > 0.05$). Untuk mengesahkan kepentingan rumpai sebagai perumah alternatif bagi antofil mangga, komposisi rumpai dan serangga dalam sepuluh, 1 m^2 kuadrat direkodkan setiap bulan selama 14 bulan pada tahun 2012 dan 2013. Min kelimpahan rumpai di kebun ini adalah berbeza ($H = 36.947$, $df = 13$, $P = 0.00$) pada setiap bulan. Antara 15 famili rumpai yang direkodkan, rumput Gramineae (44.87 %) didapati paling melimpah diikuti oleh Compositae (22.38 %) dan Acanthaceae (8.88 %) yang berdaun lebar. Hanya 17 genus antofil mangga mempunyai perhubungan yang sederhana hingga tinggi dengan spesies rumpai. Daripada jumlah itu, 10 genus adalah serangga berfaedah (pendebunga, pemangsa/parasitoid) manakala tujuh yang lain adalah perosak. Perhubungan rumpai-serangga tertinggi ($\rho = 0.705$) dikesan antara *Pieris rapae* dan *Othochloa nodosa*, *Perilampus* dan *Othochloa nodosa*, Pyralinae gen.1 dan *Gomphrena serrata*, *Episyrphus* sp.1 dan *Acalypha siamensis*. Kelimpahan 11 spesies rumpai dikawal atur oleh variasi dalam parameter persekitaran di sekitar kebun; kelembapan, suhu udara, kelajuan angin dan keamatan cahaya pada $P = 0.05$. Parameter ini juga mempengaruhi kelimpahan secara lemah hingga sederhana sembilan genus serangga pada rumpai dan 12 genus antofil mangga. Kajian ini selanjutnya menyiasat tentang kepentingan pendebunga liar dalam pengeluaran buah mangga kultivar Sala dan Chok Anan. Eksperimen yang mengecualikan pendebunga telah menunjukkan bahawa Chok Anan gagal menghasilkan putik buah tanpa kehadiran pendebunga. Pengeluaran putik buah adalah sangat rendah dalam keadaan semula jadi, 4.8% dan 3.1% bagi setiap jumlah bunga hermafrodit untuk Sala dan Chok Anan masing-masing. Dengan bantuan pendebungaan tangan, putik buah Sala telah meningkat kepada lebih 100% tetapi hanya 33% peningkatan pada Chok Anan. Sumbangan pendebunga kepada keseluruhan penghasilan buah mangga adalah dianggarkan sebanyak 53%. Lalat *Eristalinus* spp. dan *Chrysomya* spp. yang bersaiz besar membawa debunga dengan lebih cekap dan melawat lebih banyak bunga mangga berbanding pelawat bunga yang lain.

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ABSTRACT

Importance of wild pollinators in mango fruit production is widely known but their distribution in the tropics and relationships with crop plants remain poorly described. To understand the role of wild pollinators in mango production, this study investigated the distribution of flower visiting insects (anthophiles) on inflorescences of two mango cultivars, *Mangifera indica* L. cv. 'Sala' and 'Chok Anan'. Abundance of anthophiles was monitored by 15 minute hourly collection from 0800 h until 1500 h at 4-day interval from the beginning of flowering until all flowers dried up (12-28 February 2013 and 28 January 2014 – 7 March 2014). Anthophiles from 10 insect orders consisting of 79 families, 156 genera and 15803 individual insects visited mango flowers. Hymenoptera (38.54%) was the most dominant order followed by Hemiptera (34.59%) and Thysanoptera (10.32%). Chok Anan attracted significantly higher abundance of anthophiles than Sala at $P < 0.05$, $df = 264$, t = -7.490. Anthophile abundance varied following flower availability at different sampling occasions. More flying (mean abundance 34.98 ± 2.004) anthophiles visited flowers during flower anthesis compared to crawling anthophiles (24.44 ± 1.973). The gluphosinate-ammonium herbicide used to control weeds in the orchard was not toxic to the anthophiles and their abundances on Chok Anan and Sala panicles were not affected ($P > 0.05$). To verify the importance of weeds as alternate hosts for mango anthophiles, the composition of weeds and insects in ten, 1 m^2 quadrate were recorded monthly for 14 months in 2012 and 2013. There was a significant variation in monthly mean abundance of the weeds in this orchard ($H = 36.947$, $df = 13$, $P =$

0.00). Among 15 weed families recorded, Gramineae (44.87%) was the most abundant followed by the broad leaves Compositae (22.38 %) and Acanthaceae (8.88%). Only 17 genera of mango anthophiles had a moderate to high association with the weed species. Out of these, 10 genera were beneficial insects (pollinator, predator/parasitoid) while seven others were pests. The highest weed-insect association ($\rho = 0.705$) was recorded between *Pieris rapae* and *Othochloa nodosa*, *Perilampus* and *Othochloa nodosa*, Pyralinae gen.1 and *Gomphrena serrata*, *Episyrphus* sp.1 and *Acalypha siamensis*. The abundance of 11 weed species were regulated by variations in the environmental parameters in the orchard; humidity, air temperature, wind speed and light intensity at $P = 0.05$. These parameters also slightly to moderately influenced the abundance of nine genera of insects on weeds and 12 genera of mango anthophiles. This study further investigated the importance of wild pollinators in the production of mango fruits cultivars Sala and Chok Anan. A pollinator exclusion experiment had shown that Chok Anan failed to produce any fruit set in the absence of pollinators. Fruit set production was very low in natural conditions, 4.8% and 3.1% per hermaphrodite flower for Sala and Chok Anan, respectively. With the aid of hand pollination, fruit buds in Sala increased tremendously to more than 100% but only 33% increase for Chok Anan. Contribution of pollinators to total mango fruit production was estimated at 53%. Large size flies *Eristalinus* spp. and *Chrysomya* spp. were found to be efficient pollen carriers and visited more mango flowers compared to the other flower visitors.

CHAPTER 1

INTRODUCTION

1.1 Background

Pollination is one of the key processes in ecological services and it links the productivity of plant and animals in terrestrial ecosystem (Kevan 1999, Kevan and Viana 2003, Abrol 2012). Plant-pollinator interaction is one of the most important and variable mutualism in nature (Sahli and Conner 2007) that is critical for food production and human livelihood. Pollination services characterize plant communities that determine fruit and seed availability which provide tremendously important food and habitat resources for other animals (FAO 2008).

As in any horticultural region, crops show a wide range of dependence on animal pollination ranging from those that set no fruit in the absence of pollinators such as almonds and blueberries, to those that set sufficient fruit in the absence of pollinators such as olives and soybeans (Cunningham *et al.* 2002). Most of the world's staple crops (e.g. rice, wheat and maize) benefit from abiotic pollination (i.e wind, water and gravity). However, 39 out of 57 major crops (mainly vegetables and fruit) increased in fruit and seed quality through animal pollination (Klein *et al.* 2007). Approximately 73% of the world's cultivated crop such as cashew, squash, cocoa, cranberries and blueberries are pollinated by some variety of bees, 19% by flies, 6.5 % by bats, 5 % by wasps, 5 % by beetles, 4% by birds and 4 % by butterflies and moths (Abrol 2012). Garibaldi *et al.* (2011) and Hein (2009) listed tropical fruits particularly mango as high pollinator-dependence crops together with melon (*Cucumis melo*), squash and pumpkin (*Cucurbita* spp.), cashew nut

(*Anacardium occidentale*), mangosteen (*Garcinia mangostana*) and guava (*Psidium* spp.).

Globally, pollination and pollinators provide a wide range of benefits to humans. In 1992 the pollination value by honey bees in the U.S. agriculture was estimated at $1.6 - 5.7 billion (Southwick and Southwick 1992). Within 8 years, the value of pollination by honey bees increased to $14.6 billion (Morse and Calderone 2000) and reach up to $20 billion in 2010 (Johnson 2010). Meanwhile, $2-3 billion in crop pollination service was attributed to native bees such as alfalfa leaf cutting bees, bumble bees and other insects (e.g. flies, ants, wasps and beetles) (Southwick and Southwick 1992). Production of alfalfa seed alone yielded a direct monetary value of $109 million and alfalfa hay for livestock forage generated $4.6 billion per year (Morse and Calderone 2000). In Australia, value of bees' pollination was estimated to be greater than AU$ 1 billion annually with 20% of it was contributed by fruits and vegetables production. About 40% of the value came from the dairy industry as the result of pollination on pasture legumes and 25% from seed production of vegetables such as onions and carrots, as well as pasture crops, clover and lucerne (Cunningham *et al.* 2002). Previously Richards (1993) estimated the value of pollination in global agriculture alone amounted to $200 billion per year but Gallai *et al.* (2009) reported a slight drop a decade after at 153 billion euro per year ($171.3 billion). More than half (58%) of this value was contributed by Asian countries, in particular by China and India.

The use of managed pollinator in Southeast Asian agriculture seemed not to be widely practiced and consequently there is little information about animal pollination particularly insects pollination of cultivated crops. In Malaysia, most economic crops are pollinated by wild pollinators. Only carpenter bees are reared for pollination of passion fruit (*Passiflora edulis*) (Mardan *et al.* 1990). The most effective pollinator, *Elaeidobius kameronicus* Fraust (Curculionidae) was introduced from Cameroon, West Africa in 1981 to pollinate oil palm (*Elaeis guineensis*) grown in Malaysia (Syed *et al.* 1982, Basri 1984). Cameroon is also the original home for this palm and through the hard work of the weevil, Malaysia has become one of the major palm oil producers in the world. Another important crop, cocoa (*Theobroma cacao*), is pollinated by midges (Diptera: Ceratopogonidae). Mango is mostly pollinated by an assemblage of flies and other insects such as wasps, wild bees and ants (Roubik 1995). Durian, an important fruit crop of tropical Asia, is pollinated by bats (Chin and Phoon 1982, Roubik 1995) because its flower blooms when night falls and peaks at midnight parallel to the bat's flying time. Other than bats, Yumoto (2000) listed several other pollinators of *Durio* spp. in Sarawak including giant honey bees and birds.

There is evidence of recent declines in pollinator populations and because of the economic implication on reduced crop yield due to pollination failure, the issue of diminished pollination services in agricultural environment has received considerable scientific attention (e.g. Kevan and Viana 2003, Potts *et al.* 2010, Menz *et al.* 2011, Kevan and Phillips 2001, Klein *et al.* 2007, Carvalheiro *et al.* 2010, Bauer and Wing 2010, Brittain *et al.* 2010). Reduction in pollinator assemblage is mainly caused by environmental changes such as habitat loss (Carvalheiro *et al.* 2010, Ricketts *et al.* 2008) and climate change (Hegland *et al.* 2009). Application of herbicides and crop monoculture practice lead to loss of particular important plants for beneficial insects such as pollinators, predators and parasitoids but at the same time highly attractive to certain weeds and insect pests. Excessive applications of pesticides to control pests produce negative financial and environmental consequences, including damage on human health and loss of valuable pollinators.

Mango (*Mangifera indica* Linnaeus) from the family Anacardiaceae, one of the most economically important fruit crops in the tropics, is the target crop for this study. In Malaysia, mango is one of the most consumed fruits with approximately 4,565 hectares of agricultural land planted with the fruit trees (Aliakbarpour 2011). Mango plays an important part in the diet and becomes the cuisine of many diverse cultures. It is commonly grown as a garden tree in the tropics and there are over 1000 named mango varieties throughout the world (Mukherjee and Litz 2009, Bally *et al.* 2009). Young mango trees between 2 to 4 years old may flower and fruit regularly every year.

Previous studies by Sung *et al.* (2006) and Waterhouse (1993) show that mango flowers are visited by fruit bats, flies, wasps, wild bees, butterflies, moths, beetles, ants and various bugs for nectar. At the same time some of them transfer pollen to the female flowers. Usually, honeybees do not prefer mango flowers but they can act as effective mango pollinators when their numbers are high, around 3 to 6 colonies per acre (6-12 colonies per ha). However, *Mangifera indica* cv. ‘Chok Anan’ (Ding and Khairul Bariah 2013), ‘Dashehari’, ‘Langra’ and ‘Chausa’ (Mukherjee *et al.* 1968, Singh *et al.* 1962, Sharma and Singh 1970) show some degree of self incompatibility, thus cross pollination by insects is required for fruit setting. Many of the unpollinated flowers are shed or fail to set fruit, or the fruit is set but is shed when very young. For sustainable mango farming, Carvalheiro *et al.* (2010) suggested to limit the acreage of the farmland and consider practices that restore maintenance of wild pollinator-friendly areas.

Considerable efforts have been made to identify species of weeds in various orchards that are beneficial in increasing the pollination success of commercial crops. FAO (2008), Marks (2005) and Morandin and Winston (2005) reported that continuous bloom of high diversity and abundance of native trees and weeds within the fields can attract beneficial insects in particular the pollinators because they provide uninterrupted source of food to these insects. However, abundant native flowers also support populations of pests and become competitors to farmed crops for nutrient, space and pollinators (Abrol 2012, Aini *et al.* 2011). Meanwhile, incessant availability of attractive native flowers affect pollinator foraging decision among available floral resources thus reduces visitation to commercial crops (Brittain *et al.* 2010, Totland *et al.* 2006). Therefore, in pollinator conservation and management program, maintenance of farmscapes and identification of beneficial native floral resources deserve high priority.

As a measure for facing the global crisis for crop pollination, more data on pollinator-plant relationships are urgently needed. A better understanding of pollination processes and the cause of disruption are sorely needed to assess pollinator limitation. Methods to improve pollinator visitation to commercial crops also need to be identified. According to FAO (2008) there is a paucity of attention to pollination services at all levels of formal and informal education. Due to the lack of time and facilities, many of the studies on tropical crop pollination were preliminary in nature.

Though pollinators are known to provide essential services to ecosystem functions, changes in their distribution and plant-pollinator relationship remain poorly described. Therefore, this study was proposed to investigate the pollinator's communities that pollinate mango flowers and to understand their relation with other organisms, their biology and importance to mango production. The results from this study would provide a useful guideline for management recommendations that may benefit insect community with concomitant improvement in pollinator dependent crop production in Malaysia.

1.2 Objectives

In view of the importance of pollinators in crop production, this research emphasized on its ecology and pollination activity in a mango orchard focusing on the following objectives:

1. To identify flower visiting insects (anthophiles) and potential mango pollinators on mango panicles and weed around the orchard.
2. To evaluate the influence of environmental parameters on diversity and abundance of anthophiles and mango pollinators.
3. To assess pollinator performance based on the number of mango fruit sets produced after pollination treatments.
4. To investigate on the pollination activities of dominant pollinator species in a mango orchard.

CHAPTER 2

LITERATURE REVIEW

2.1 Pollination and pollinators

Pollination is simply the transfer of pollen from the male anther to female stigma of another or the same flower. Pollination can occur through a wide range of mechanisms that ensure an appropriate balance in the genetic makeup of the species. Natural agents of pollination can either be abiotic or biotic. Abiotic pollination occurs through wind, water (Corlett 2004) or gravity (Abrol 2012). Wind pollination is the dominant type of abiotic pollination especially prevalent in several plant families, including grasses (Poaceae) and sedges (Cyperaceae). Biotic pollination is carried out by animals (Kevan 1999) such as insects, bats and birds.

Animals that assist plants in their reproduction (pollination) are called pollinator. An active pollinator constantly carry enough pollen for deposition on a stigma of a given plant species (Borkent and Schlinger 2008). Insects have the potential for explosive population increase therefore appear to be ideal pollinator for most of the plant species. Insects such as bees and particularly honey bee (genus *Apis*) are primary pollinators of most managed crops and wild plants (Hein 2009, Potts *et al.* 2010).

The goals of pollination differ between pollinators and flowers. Pollinators are likely to feed on nectar or pollen from a variety of different flowers while flowers must transfer pollen to another of the same species. Flowers thus benefit most from either a specialist pollinator or by attracting the greatest number of pollinators possible to increase the chances of successful fertilization. Most pollinators receive food in the form of pollen or nectar, but some bees also use waxes and resins from flowers to build their hives (Triplehorn and Johnson 2005, Michener 2007). Zimmermann *et al.* (2006) have discovered that male euglossine bees use volatile compounds from orchid flowers as "perfume" to attract mates. Other insect species, such as yucca moths, lay their eggs within the yucca flowers they pollinate, and some of the seeds produced are consumed by the developing moth larvae (Pellmyr 2003).

Hymenoptera including honeybees (*Apis* spp.), wild bees, wasps and ants has long been associated with pollination, visiting more flowers because they actively collect pollen and nectar for provision to their young. Among the pollinators, bees are one of the most important and specialized groups (Danforth *et al.* 2006). Over 25000 species of bees are found around the world, which includes honey bees, bumble bees, stingless bees and solitary bees (Abrol 2012). The most common solitary bees are alfalfa leaf cutter bees *Megachile rotundata*, mason bees *Osmia conifrons* and alkali bees *Nomia melanderi*. In a large plant of the genus *Ficus* (fig) (Moraceae), almost every fig species has a different wasp species (Agaonidae) as a pollinator. In *Ficus macrophylla*, the winged female wasps, *Pleistodontes froggatti*, are the pollen carriers whereas the wingless males are not involved in the pollination process (Early 2000). Anthophilus ants are not likely to facilitate pollination due to their hard, generally smooth and small size body. Such morphology is devoid of contact with anthers and stigmas during flower visit. However, genuine cases of ant pollination are shown by three genera of ants (*Iridomyrmex*, *Meranoplus* and *Rhytidoponera*) that feed on *Microtis parviflora* (Orchidaceae) and effectively pollinate 70% of the blossoms within 3 day anthesis (Jones 1975).

Fly pollination (Myophily) usually involved families with short mouthparts. Abrol (2012) listed at least 12 families from the suborder Nematocera known to contain anthophilous taxa. At least seventy-one families of Diptera consist of flower-visiting flies that pollinate or visit approximately 555 flowering plant species (Larson *et al.* 2001). Specialized pollination occurs between mosquitoes and highly evolved plant *Habenaria* orchids (Kevan *et al.* 1993). Cocoa (*Theobroma cacao*) the seeds of which are the main ingredient for making chocolate is pollinated by several midges from the Family Ceratopogonidae and Cecidomyiidae (Free 1993, Roubik 1995). Among the short-horned flies (suborder Brachycera), there are many records of flower visiting species. Sajjad and Saeed (2010) documented 51 species of flowering plants in 28 families were visited by hoverflies in Southern Punjab, Pakistan. *Parkinsonia aculeate* and *Mangifera indica* are agricultural and non-agricultural plant species respectively, most preferred by syrphid flies.

Beetles are documented as pollinators of, or visitors to, a diverse array of angiosperms in the Oriental region (Corlett 2004). Almost all beetles that visit flowers in the region belong to the huge suborder, Polyphaga. Most records refer to plants of the families Annonaceae, Myristicaceae, Dipterocarpaceae, Araceae or Palmae. The plants that require beetle pollination are usually equipped with a strong odor and flower heat production (thermogenic plants) probably to volatilize the fragrance (Maeto *et al.* 1995). Curculionids, staphylinids and chrysomelids have been reported as pollinators of three species of *Knema* (Myristicaceae) and one of *Gymnacranthera* (Myristicaceae) in Sarawak (Momose *et al.* 1998). The main pollinator of *Philodendron solimoesense* (subgenus Meconostigma) of French Guiana is *Cyclocephala colasi* (Scarabaeidae, Dynastinae) (Gibernau *et al.* 1999). *Homalomena propinqua* (Araceae) in the understorey of the forest in Sarawak is predominantly visited by a scarabaeid and chrysomelid beetles which chose to shelter and mate in its flower chamber (Kato 1996). The world's largest flower, the stinking corpse lily (*Rafflesia* sp.), is pollinated by a carrion beetle (Kevan and Viana 2003). Anthicid beetle, *Macratria griseoselata* is found specifically in the flower tubes of *Mussaenda parviflora* (Kato 2000). Other example is pollination of oil palm in West Africa, Malaysia and Indonesia by *Elaeidobius kamerunicus* (Eardley *et al.* 2006).

Pollination by Lepidoptera has been popularly modeled by a hawk moth (Sphingidae) with a highly specialized long, thin and flexible proboscis. Flowers visited by hawk moths open in the evening and are extremely fragrant (Abrol 2012). Momose *et al.* (1998) found that hawk moth pollinated *Barringtonia* (Lecythidaceae), the nocturnal brush flower in lowland dipterocarp forest of Sarawak. In addition, Kato (2000) found two other plant species; *Cerbera manghas* and *Clerodendron trichotomum* on Amami Islands in the Ryukyu Archipelago that were also pollinated by hawk moths. Other lepidopteran families such as Geometridae were the main pollinators for *Dipterocarpus pachyphyllus* (Momose *et al.* 1998) and Pyralidae were pollinators for *Gnetum gnemum* (Kato *et al.* 1995) in the understorey of Sarawak forest.

Thrips (Thysanoptera) are important pollinator, but they are tiny, short-lived and lack traits that are deemed essential to be an effective pollinator (Corlett 2004). The role of thrips in pollinating *Shorea* species in Malaysia has been reported as early as in 1981 (Appanah and Chan 1981, Appanah 1993). Adult thrips visit the open flowers to feed on pollen and flower tissues, and accidentally carried sticky pollen on their bodies. Dioecious pioneer tree *Macaranga hullettii*, a common species found in Southeast Asia are pollinated by thrips *Neoheegeria* in Malaysian rainforest (Moog *et al.* 2002).

Others insects such as true bugs are common as flower visitors but not often considered as pollinators. Their presence is usually destructive to plants but sometimes they might transfer pollen among visited flowers (Corlett 2004). Appanah (1987, 1993) reported that Miridae is a possible pollinator for *Shorea* section *Shorea* and Cicadellidae for *Shorea* section *Brachypterae*. Meanwhile, Moog *et al.* (2002) suggested that these bugs may be involved in the pollination of *Macaranga*. Scavenger cockroaches also visit flowers and may become primary pollinators of certain Annonaceae plant such as *Uvaria elmeri* (Nagamitsu and Inoue 1997). In Sarawak (Malaysia) blattellid cockroaches visited both male and female inflorescence of *Artocarpus odoratissimus*, and some of them carried pollen on their bodies (Momose *et al.* 1998).

Large size vertebrates are not well known to pollinate flowers and hence were overlooked as a pollinating agent. Ornithophily (birds) and therophily (mammals) are important vertebrate pollinators for many plants. Bumrungsri *et al.* (2009) identified that fruit bats, especially *Eonycteris spelaea*, are the major pollinators of durian in southern Thailand. This bat is the most common nectarivorous species in Thailand, flying long distances each night. Apart from bats, Yumoto (2000) noted that nectarivorous birds such as hummingbirds and sunbirds are pollinators of three species of *Durio* in a tropical rainforest of Sarawak. Eardley *et al.* (2006) documented that hummingbirds in the Western Hemisphere and sunbirds in the Old World are pollinators of several native plant species and contribute to the pollination of crops such as papaya and okra. Endemic passerine bird of New Zealand, Tui

(*Prosthemadera novaeseelandiae*) is one of the largest members of the diverse honeyeater family and 'specialist' pollinator of bright red mistletoe (*Peraxilla tetrapetala*) flowers (Robertson *et al.* 2005). About 528 species of angiosperms are pollinated by nectar-feeding bat and at least 2000 species of birds feed on nectar or pollen (Abrol 2012).

Mammals, like the black and white ruffed lemurs found in eastern rainforest of Madagascar are linked with the traveller's palm tree when they accidentally transferred pollen from one bloom to another during their movement to feed on nectar (Kress *et al.* 1994). Carthew and Goldingay (1997) reported that marsupials, primates and rodents pollinate some native plants in Australia, Africa and South and Central America. Other animals like lizards also feed on nectar and fruits of plants (Abrol 2012). Traveset and Saez (1997) found that Lilford's wall lizard (*Podarchis lilfordi*) is the true pollinator for *Euphorbia dendroides* based on the increasing fruit and seed sets of the plant. Eifler (1995) suggested that the distribution of geckos from the genus *Hoplodactylus* is influenced by the pattern of nectar availability and hence a potential plant pollinator.

2.2 Pollinators in agricultural ecosystem

Honey bee is more "generalist" compared to other wild bee hence being domesticated for many commercial crops (Abrol 2012, Woodcock 2012, Bohart 1972). Honey bees are important pollinator as they actively seek out flowers with pollen, unlike pollinators such as bats and hummingbirds which are primarily interested in nectar. They also live in large, well organized colonies of around 50,000 to 60,000 workers. In addition to pollination, honey bee colonies are managed to produce surplus honey, beeswax, royal jelly and propolis which are all marketable products (DeGrandi-Hoffman, 1987).

Apart from being a generalist pollinator, honey bees fail to efficiently pollinate alfalfa, the world's most important forage crop. To overcome the situation, pollination by wild bees has been studied intensively on the crop (Bohart 1972, Woodcock 2012). The alfalfa leafcutter bee (*Megachile rotundata*) is an important pollinator for seed producing alfalfa in western United States and Canada (Abrol 2012, Woodcock 2012). In North America, several bees have been investigated for their suitability as pollinator of greenhouse tomatoes. Bumble bees (*Bombus impatiens*) are produced commercially in Ontario, Canada (Woodcock 2012, Morandin *et al.* 2001) and *B. occidentalis* in western North America (Dogterom *et al.* 1998). Up to 50 bumble bees colonies were used per hectare during a tomato growing season with the value of pollinated crops estimated to be 12000 million per year (Abrol 2012).

In Japan, *Osmia cornifrons* (Hymenoptera; Megachilidae) has been successfully managed for apple pollination since 1958 in northern and central Honshu (Bohart 1972). This bee is an effective pollinator for rosaceous fruit such as almond, cherry, peach, pear, plum and apple because the flowering time of the crops coincides with emergence of the bee (Abel and Wilson 1998). Bosch (1994) has demonstrated that another species of *Osmia*, *O. cornuta* has a great pollinating potential in almond orchards. Large passion flower (*Passiflora edulis*) in Central America and Asia are efficiently pollinated by large bees such as *Ptiloglossa* and *Xylocopa* (Eardley *et al.* 2006, Roubik 1995).

Non-bee pollinators include flies, beetles, moths, butterflies, wasps, ants, birds, and bats. According to Rader *et al.* (2009) non-bees are less effective pollinators than bees per flower visit but they make more visits, thus these two factors compensate for each other, resulting in pollination services rendered by non-bees that are similar to those provided by bees. More than 100 cultivated crops are regularly visited by flies and depend largely on fly pollination for abundant fruit set and seed production (Ssymank *at al.* 2008). In India, some large fly species such as *Lucilia* sp. (Calliphoridae) and *Sarcophaga* sp. (Sarcophagidae) has been reared in mango orchard to assist mango pollination (Sharma *et al.* 1998). Meanwhile, in United States, calliphorid flies are raised commercially to pollinate crops including canola, sunflowers, buckwheat, garlic, lettuce and peppers (Ssymank *et al.* 2008).

As a biotic process, pollination has both commercial and ecological value (Abrol 2012). The value of busy pollinators (which include insects, birds, bats and other animals) is immeasurable. Animal pollinators including insects increase the output of 87 leading food crops worldwide with 75.6% of global primary food crops require some level of animal pollination and 35% of crop production is strictly pollinator dependent (Klein *et al.* 2007). Gallai *et al.* (2009) estimated the value of animal pollination services globally to be € 153 billion (~$200 billion) and Bauer and Wing (2010) justified that in the United States, honey bee pollination alone amounted to $14.6 billion. Meanwhile, according to Potts *et al.* (2010), approximately 75% of all crops used as human food worldwide are pollinated by wild bees. In apple production alone, it is estimated that through pollination services, wild insects including wild bees contribute a total of £36.7 million (~$51.27 million) per annum to Cox and Gala production in the UK (Garrat *et al.* 2014).

2.3 Possible causes of pollinator decline

In an FAO report (FAO 2008), the number of honey bee colony has steadily increased globally over the past 50 years but it has plummeted on regional scale especially in Europe and North America. During the same period, most wild bee colonies have been lost. Many European butterflies are under serious threat owing to changing land-use and agriculture intensification. Among mammalian and bird pollinators, at least 45 species of bats, 36 species of non-flying mammals, 26 species of hummingbirds, seven species of sunbirds and 70 species of passerine birds are considered threatened or extinct (FAO 2008).

Many of the previous research papers discussed on global pollination crisis (e.g. Potts *et al.* 2010, Kevan and Viana 2003, Bauer and Wing 2010) and importance of pollinators in changing landscapes (e.g. Calvalheiro *et al.* 2010, Klein *et al.* 2007, Ricketts *et al.* 2008). Pollinator decline and loss of pollination services have become political, media and scientific issues worldwide (Mayer *et al.* 2011). It received widespread attention in 2006 when a popular press reported on the mysterious disappearance of managed honey bee colonies across the United States (Bauer and Wing 2010).

There are many factors involved in pollinator decline. Rapid growth of human population leads to reduction of natural habitats through an increasing demand for food-producing areas, urbanization and other land-use practices, putting pressure on the ecosystem service delivered by wild pollinators. The biggest threat that caused pollinator decline is loss of important resources such food, foraging ground, reproduction and nesting area (Klein *et al.* 2007) mainly the results of habitat alteration, fragmentation and degradation due to increasing agricultural intensification. According to FAO (2008), loss of any of these requirements can cause pollinators to become locally extinct. It has been suggested that an agriculture crisis in pollinator dependant crops is only likely to occur in areas where little natural habitats remain (Carvalheiro *et al.* 2010). Distance of agricultural land from natural habitat negatively affects the richness and abundance of pollinator especially wild bees. Visitation rate of native pollinators especially the tropical species also declined with increasing distance from natural habitats (Ricketts *et al.* 2008).

Most ecosystems have been simplified through human influence and subsequently cause negative impact on pollinator populations. Fragmentation of land due to monoculture practices lead to poor species and low density of native bee communities (Winfree *et al.* 2007). The effect is more significant in solitary, parasitic and specialized bees (Richards 2001). Habitat fragmentation accelerates the extinction of local plants through inbreeding and genetic drift and loss of floral that provides nesting resources due to excessive use or inappropriate application of agrochemicals (e.g. insecticides, herbicides and fertilizers) (Kevan 1999, Donaldson 2002). Pollinators display a range of responses to habitat fragmentation by increasing as well as decreasing their population corresponding to their dispersal ability and habitat specificity (FAO 2008).

Introduction of alien species (plant, pollinator, pest and pathogen) also calls for pollinator decline. Alien plants have been introduced to farmland as additional pollen and nectar sources for pollinator during non-flowering season. This practice has positive effects on generalist pollinator but disrupt native plant-pollinator interaction (Traveset and Richardson 2006). Meanwhile, competition between native and alien pollinators can lead to problem such as genetic dilution between managed and wild bees which can interbreed leading to extinction of local sub-species (Potts *et al.* 2010). For example, all subspecies of honey bee *Apis mellifera* can interbreed or hybridize. Consequently, hybridization between the introduced African honey bee *A. mellifera scutellata* and the European bees such as *A. mellifera mellifera* became frequent as the African bees moved into areas previously occupied by the European bees (Schneider *et al.* 2003).

The phenomenon of climate change may potentially be one of the most severe treat to pollinator biodiversity which affects the spatial-temporal dynamics of plant pollinator interactions (Mayer *et al.* 2011, Kjohl *et al.* 2011). Spread of pest and pathogen such as parasitic mites *Varroa jacobsoni*, *V. destructor* and *Acarapsis woodi* (Winfree *et al.* 2007, Klein *et al.* 2007) are major causes of honeybee declines all over the world. The emergence of serious and widespread diseases has made it clear that native pollinators need to be protected and sustainably managed since they provide potential insurance against the loss of honey bee (Winfree *et al.* 2007). Climate change also induces mismatch in temporal and spatial co-occurrence and morphological and physiological interdependences of plant and pollinators and disrupted their interaction.

Combination of multiple drivers and pressures all together might cause unprecedented decline of domesticated honey bees. This phenomenon is termed Colony Collapse Disorder (CCD). There does not appear to be any single pest or pathogen responsible for this phenomenon (Bauer and Wing 2010). Due to variation in crop species and heterogeneity of agricultural landscapes, the vulnerability to pollinator decline varies widely among the different continents and regions (Gallai *et* *al.* 2009). Except for the Antarctica, FAO (2008) has reported on pollinator declines in at least one region or country in every continent.

2.4 Pollinator in Malaysian agricultural ecosystems

Currently, the majority of studies on pollinator in Malaysia focus more on wild pollinator populations in their natural environment especially on the ecological value of stingless bees, wild honey bees, flies, thrips and Polyphaga beetles (Corlett 2004). Eltz *et al.* (2002) investigated the population of stingless bee in lowland dipterocarp forest in Sabah. Samejima *et al.* (2004) assessed the effect of human disturbance on a stingless bee community in a tropical rainforest, in Sarawak and Salim *et al.* (2012) surveyed stingless bee in Virgin Jungle Reserves (VJRs) located throughout Peninsular Malaysia.

The first detailed report on pollination of wild plants in Malaysia was provided by Appanah and Chan in 1981. They investigated pollination of six co-occurring species of *Shorea parvifolia* section *Mutica* by thrips at Pasoh forest. Two decades later, Moog *et al.* (2002) documented pollination of the dioecious pioneer tree *Macaranga hullettii* in a Malaysian rainforest by its major pollinator, a thrips species, *Neoheegeria* sp. (Phlaeothripidae). Very recently, Fialla *et al.* (2011) also reported that Thysanoptera (thrips) was the most abundant insect pollinator of 20 *Macaranga* species in various regions of peninsular Malaysia and Borneo. A more sophisticated pollination was investigated by Tan and Nishida (2000) and Tan *et al.* *(*2002). They found that an epiphytic orchid, *Bulbophyllum patens* produced a specific fragrance, zingerone to attract males of several *Bactrocera* species to pollinate their flowers. Ismail *et al.* (2010) discovered the polllination of a beneficial plant, *Curculigo latifolia* (Hypoxidacea), (locally known as Lemba) which has high potential as a source of low calorie sweetener by black ant and bee

Research of pollination and its application in crop production in Malaysia progresses very slowly compared to the achievements in the western countries. The earliest study on crop pollination in Malaysia started when the African pollinating weevil *Elaeidobious kamerunicus* was introduced from Africa into the oil palm growing regions of Asia and the Pacific in the early 1980s (Syed *et al.* 1982, Caudwell *et al.* 2003). *Elaeidobious kamerunicus* was introduced in Malaysia under the quarantine care of the Department of Agriculture between July and December 1980 (Kang and Karim 1982). The weevil was released into two estates in Johor and Sabah in February and March 1981. Basri (1984) found that the introductions of *E. kamerunicus* into Malaysia had tremendously increased oil palm fruit set from an average of 52% to 71%. Over the years *E. kamerunicus* became more efficient and and subsequently increased oil palm production in Malaysia and Indonesia to become the world's leading palm oil producing countries (Eardley *et al.* 2006).

Other studies on crop pollination in Malaysia were conducted by Mardan *et al.* (1990) on passion fruit (*Passiflora* sp.) and Yumoto (2000) on *Durio* spp. Crops such as starfruit (*Averrhoa carambola*), snake fruit (*Salacca zalacca*), guava (*Psidium guajava*) and watermelon (*Citrullus lunatus*) benefit from pollination by wild pollinators (Free 1993). Heard (1999) noted that large numbers of two wild bee species, *Trigona thoracica* and *Apis cerana* visited flowers of carambola in orchards in Malaysia and carried large pollen loads on their bodies. Although many evidents proved that pollinators are important in the pollination of Malaysian crops, managed pollination such as by honey bees has not been fully developed. Pollination of crops depends mostly on wild pollinators. According to Ricketts *et al.* (2008), pollination of tropical crops primarily by social bees (e.g. Meliponines) are the most susceptible to pollination failure due to changes in usage of the surrounding lands. Therefore understanding of pollinator ecology is very crucial for future benefit.

2.5 Effect of environmental factors (weather) on pollinators foraging activities and abundances.

Environmental factors strongly affect the foraging activity of pollinating insects (Herrera 1995b, Vicens and Bosch 2000) because their body temperature rises and falls in tandem with the environmental temperature (Triplehorn and Johnson 2005). According to El-Moursy *et al.* (1999), climatic factors plays an important role in determining the occurrence and timing of activities and hence the frequency of insect visits to flowers. Air temperature, light intensity, humidity, wind speed as well as precipitation influence foraging activities and pollinator performance to various extents.

Tropical insects live within narrow span of suitable temperature and they are relatively sensitive to temperature changes. However, those living at higher latitudes have broader thermal tolerance and are living in cooler climates than their optimum limit (Kjohl *et al.* 2011). The environmental temperature always becomes the turning point because flying pollinators usually rise their body temperature above that of the environment. Temperature of flight muscles must be maintained above certain point to produce energy necessary for flight (Triplehorn and Johnson 2005). When the air temperature goes down to about 14°C, honey bees remain in the hive and they use their thoracic muscles to maintain the temperature of the cluster and maintain ventilation. Ventilation of bee nests through fanning activity has long been recognized as a social thermoregulatory measure. However, in hives with stable temperature, an increase in CO_2 also induced fanning behavior in some bumble bees (Weidenmüller 2004).

According to Mardan and Kevan (2002) the broods of honey bees *Apis dorsata* and *A. mellifera* are highly sensitive to high temperatures and somewhat less sensitive to low temperatures. Adult workers of *A. dorsata* survived well at temperatures ranging from 26 to 36 ^{o}C.

Vicens and Bosch (2000) found that initiation of foraging activity of *Osmia cornuta* (Megachilidae) is limited by temperature. Females of *O. cornuta* start foraging at lower temperatures (10-12^{o}C) on days with clear sky. When temperature is unfavorably cool, these bees waited until the temperature rises to above “normal” levels. *Apis mellifera* on the other hand is fully active at temperatures higher than 12-14^{o}C. Similarly, foraging behavior and flower visitation rate of *Andrena bicolor* (Andrenidae) were also temperature dependent (Herrera 1995a). It foraged in *Narcissus longispathus* flowering patches only on sunny days with air temperature of more than 12-13^{o}C.

High temperatures also have a strong negative influence on the number of pollen loads and positively correlated with nectar loads collected by pollinators (Fidalgo and Kleinert 2010). This statement is supported by a previous study by Gilbert (1985) where two pollen specialists (*Syrphus ribesii* and *Episyrphus balteatus*) started feeding on pollen when temperature was above 12^{o}C but only took nectar at higher temperature of 20^{o}C.

Light intensity in relation to cloud-cover also influence foraging activity of pollinators. Study by Herrera (1995b) showed positive influence of light intensity on five out of 10 pollinator species studied. The muscoid flies spend the cool nights on the flowers. Consequently, they show greater activity at the lowest temperature and light intensity of the day (Vicens and Bosch 2000). Gilbert (1985) noted that certain species of syrphids remain active even at low temperature in the presence of sunlight.

Gilbert (1985) also reported that nectar feeding occurred more often on flower under the sun than flower in the shade. However, two species of hoverflies in his study showed opposite reaction to light intensity. *Melanostoma scalare* remains active under low light intensity whereas *Metasyrphus corolla* appears only when light intensity is high. Light intensity influenced the behavior of pollinators in collecting nectar and pollen loads. More nectar (loads) is collected at higher light intensity (Fidalgo and Kleinert 2010).

Some insects such as bees have a well-developed humidity sensor (Triplehorn and Johnson 2005). A study by Puškadija *et al.* (2007) demonstrated that the most frequent visit of honey bee on sunflower inflorescence was recorded at 65-75% humidity. Similar to light intensity, humidity plays an important role in controlling the uptake of pollen and nectar by pollinators. Fidalgo and Kleinert (2010) and Gilbert (1985) reported that the percentage of nectar loads collected is negatively correlated with relative humidity. The incidence of nectar feeding by *Melipona rufiventris* (Hymenoptera: Apidae) and *Episyrphus balteatus* (Diptera: Syrphidae) increased to a peak near midday when the relative humidity is low (<60%).

Meanwhile, the number of pollen loads increased as relative humidity rose with peak pollen collected during the early morning (Fidalgo and Kleinert 2010 and

Gilbert 1985). However, Peat and Goulson (2005) recorded that bumblebee *Bombus terrestris* collected more pollen in the middle of the day when the humidity was low to avoid water-droplet on vegetation, which would make grooming the pollen into the curbiculae difficult.

According to Vicens and Bosch (2000), all insect groups studied were active at wind speeds of up to 6 m/s. Three pollinator species, *O. cornuta, A. mellifera,* and the syrphids were still active at wind speed above this value. *O. cornuta* was able to forage under strong wind conditions reaching maximum values of 50 km/h as well as under light rain. Meanwhile, honey bee was more sensitive to wind and precipitation than *O. cornuta*. Abrol (2012) observed that the flight activity of honeybees stop completely when the wind speed exceeds 25 mph. However, based on the study by Puškadija *et al.* (2007), honey bee was absent from the study sites during rainy days. In general, strong winds together with heavy precipitation have a negative impact on pollinators visit. Hot wind also reduces nectar secretion, thereby reducing the favorable climate for pollen germination (Abrol 2012).

2.6 Measuring pollinator performance

Pollination requirements vary between plant species or cultivars depending on geographic location, availability of natural habitat and use of pesticide (Kremen *et al.* 2002). Measuring pollinator performance has become increasingly important with emerging needs for risk assessment in conservation and sustainable agriculture (Ne’eman *et al.* 2010). Different characteristics and behavior of pollinators influence their ability to effect pollination (Horsburgh *et al.* 2011). Multiple pollinators vary in visitation rates, pollen removal and deposition and spatial and temporal distribution (Sahli and Conner 2007). Spears (1983) stated that measurement of pollinator effectiveness can be divided into direct (using seed set produced by a plant population in response to pollinator visits) and indirect measurements (rely on the pollen carried by the visitor) which are further refined by determining the relative abundance of visitors, visitation rates to flowers, and relative amounts of pollen transferred to stigmas.

Measuring pollen deposition onto stigmas by insects is useful to assess pollinator effectiveness, but it can be unpredictable and time-consuming as insects must visit test flowers. In contrast, the measurement of pollen grains directly from flower-visiting insects shows potential as a quicker and easier technique to assess pollinator effectiveness because the insects can be directly collected from flowers (Howlett *et al.* 2011). Amount of pollen that an insect carries can provide useful information on the foraging behavior, pollinating ability and ecology of the insect (Borkent and Schlinger 2008, O'Neill and O'Neill 2010).

The most effective insect pollinators are always present in high numbers with high visitation rate as well as frequently contacting the stigma and transferring many pollen grains (Rader *et al.* 2009). Several research papers have discussed the effectiveness and efficiency of pollinators in the pollination of various plants. Differences in pollination effectiveness of birds and insects visiting *Banksia menziesii* (Proteaceae) was assessed by Ramsey (1988). The bees visited ten times more frequently than birds but only deposited 25% of the pollen on stigma thus less fruit set compared to inflorescences visited by birds which are directly in contact with pollen. According to Larsson (2005), pollination effectiveness of specialist pollinator *Andrena hattorfiana* (Andrenidae) is far superior compared to generalist such as *Bombus lapidaries* (Apidae) as shown by pollination of gynodioecious herb