

OBSERVATION OF FLOWER VISITORS TO DETERMINE THEIR POTENTIAL ROLE AS POLLINATORS OF *Ixora coccinae* AND *Ruellia simplex*

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Abstract: This study aimed to determine the potential pollinating agents of *Ixora coccinae* and *Ruellia simplex* at Universiti Malaysia Terengganu (UMT) campus based on the foraging activities of the flower visitors. The diurnal observations (0700 hr to 1800 hr) were conducted for a total of 144 hours and 96 hours for *I. coccinae*, for *R. simplex* respectively. The flower visitor activities (landing or hovering at the flowers) and the time spent at the flowers however were recorded for only the first 10 minutes of every hour between 0700 hr and 1000 hr for four days. A total of 383 flower visitations was recorded by 17 insect species and a bird species, the olive-backed sunbird (*Cinnyris jugularis*). For *I. coccinae*, the most common visitors were *Apis cerana*, *Heterotrigena itama* and *Xylocopa confusa*, in which all species were found to show only landing behaviour to forage at the flowers, and the time spent foraging at the flowers was not significantly different between the species (Kruskal-Wallis test, $H = 1.323$, $df = 2$, $p = 0.055$). For *R. simplex*, the most common visitors were *A. cerana* with 100% landing, and *C. jugularis* which recorded 80% landing and 20% hovering during their visits to the flowers. Between the two, *A. cerana* recorded significantly longer time spent at the flowers as compared to *C. jugularis* (Mann-Whitney test, $U = 17.355$, $p < 0.001$). Based on the visitation rate and feeding behaviour of the flower visitors, *A. cerana*, *H. itama* and *X. confusa* were the potential pollinating agents for *I. coccinae* while for *R. simplex*, *A. cerana* showed higher potential to be a pollinating agent as compared to *C. jugularis*. Therefore, this study highlighted the importance of recording the behaviour of each flower visitor to determine the relative contribution to the pollination success of the plant species visited.

Keywords: *Apis cerana*, *C. jugularis*, flower visitors, landing, pollinating agents, time spent.

Introduction

Flower visitors come to flowers to feed on nectar and other flower parts. While feeding on the flowers, flower visitors might contact the stigma and anthers of the flowers and therefore act as pollinating agents by transferring the pollen to the stigma of the flowers for fertilisation. Although pollination could occur by the help of wind, pollen transfer for the majority of tropical flowering plants was conducted by the animals as vector (Stein & Hansen, 2011; Rech *et al.*, 2016). Further, Regal (1982) showed that pollination by wind is uncommon in tropical rainforest due to the dense structure of the forest which reduces the wind speed. In this mutualistic interaction, both flowering plant species and the

animal pollinating agents were benefitted in terms of pollination service and the reward for the animal agents (Glover, 2007; Jedrzejewska-Szmek & Zych, 2013).

Pollination by animals in flowering plants is approximately 87% worldwide (Ollerton *et al.*, 2011). Pollination by reptiles such as by lizards is more common in the island than in the mainland (Olesen & Valido, 2003), while pollination by rodents occurs for geoflory flowers, in which flowers are at the ground level (Johnson & Pauw, 2014). Insects and birds, however, are among the most common pollinating agents. For example, Cronk and Ojeda (2008) stated that birds from Nectariniidae family which are

spiderhunters and sunbirds are great pollinating agents in Asia and Africa. Insects from the order of Coleoptera, Diptera, Hymenoptera and Lepidoptera are among the most common pollinating agents (Dobson, 2006). The stingless bees (Hymenoptera) for example, are the new effective commercial pollinating agents especially for the crops (Slaa et al., 2006) in which apiary industry has emerged.

The two flowering species selected in this study, *I. coccinae* and *R. simplex* are among the most cultivated garden and landscape plants in the Asian region. *Ixora* is a genus from Rubiaceae family with approximately 500 species (De Block, 1998), including many cultivars and hybrids (Rajaseger et al., 1997; Mouly et al., 2009). *Ixora coccinae* is a native shrub of Southern India, Bangladesh and Sri Lanka, but it is widely planted in other Asian regions for its showy corollas (Mouly et al., 2009). This evergreen shrub can grow up to 3m in height and flowers can be found throughout the year. The flowers are small tubular, bright red and are in a dense rounded cluster. There are about 2-5 flowers in a cluster arranged in opposite and articulate axes. The flowers are erect at a terminal position for ease of access by the flower visitors.

Ruellia is also a highly diverse genus from the family of Acanthaceae, with ca. 300 species (Wasshausen & Wood, 2003). *Reullias* are popular ornamental plants with various corolla colour from red, yellow and purple, although purple and red are the most common from the anthocyanin's pigments produced by the plants (Bloom, 1976). *Ruellia simplex* is an evergreen perennial plant which is native to Mexico and South America. The plants are about 1m tall and the flowers are trumpet-shaped with purple corollas.

Previous studies reported Lepidoptera (butterflies and moths) as pollinating agents for

the genus *Ixora* (Nilsson et al., 1990; Kato et al., 2008; Duara, 2014), while wide array of insects (butterflies, moths, bees, beetles, hemipteran), birds and event bats (Trip & Manos, 2008; Faria & Araujo, 2010; Hawkeswood & Sommung, 2016) were reported as pollinators of the genus *Ruellia*. The conclusion of these studies, however, was made from visitation of these insects and vertebrate faunas to the flowers to feed. Feeding behaviour of floral visitors however determines their role as legitimate, effective or efficient pollinators (Fleming & Sosa, 1994). For example, some floral visitors are only nectar robbers or pollen thieves, in which these floral visitors are only taking pollen and nectar without making contact with the reproductive organs, thus precluding the possibility of pollination occurring (Inouye, 1980). Therefore, in this study, the pollinating agents of *Ixora coccinae* (jungle geranium) and *Ruellia simplex* (Mexican bluebell) were determined from the feeding activities (*viz.* visitation frequencies and time spent at the flowers) of the flower visitors at Universiti Malaysia Terengganu campus, Terengganu.

Materials and Methods

Study site

The study was conducted at Universiti Malaysia Terengganu (UMT) campus in the Kuala Nerus District, located 25 km from the city of Kuala Terengganu. The two flowering plants selected in this study have been planted abundantly on the campus (Figure 1). Observations of *I. coccinae* were conducted behind *Bina Syarahan* building (5°24'47.49"N, 103°5'13.47"E,) while *R. simplex* was observed in front of the School of Marine and Environmental Sciences building (5°24'42.93"N, 103°5'7.74"E, Figure 2). At the study area, there were nine trees and 50 plants for *I. coccinae* and *R. simplex* respectively.

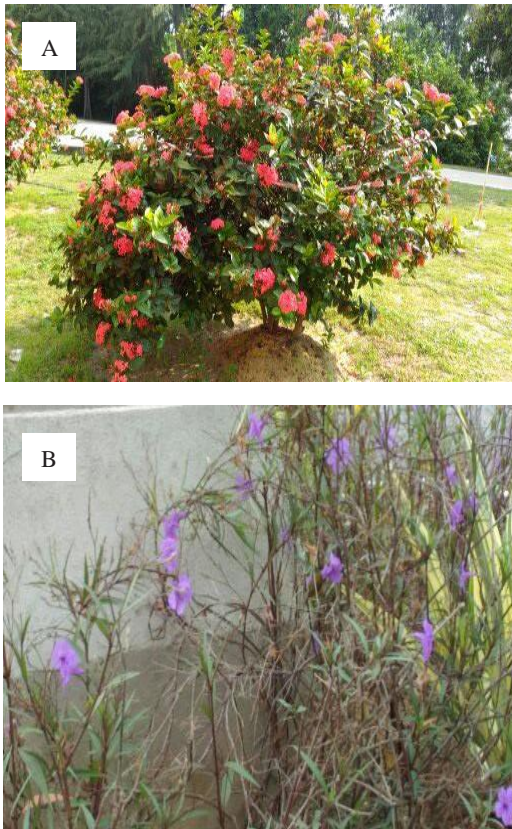


Figure 1: The flowering plants observed in this study: (A) *Ixora coccinae* and (B) *Ruellia simplex*

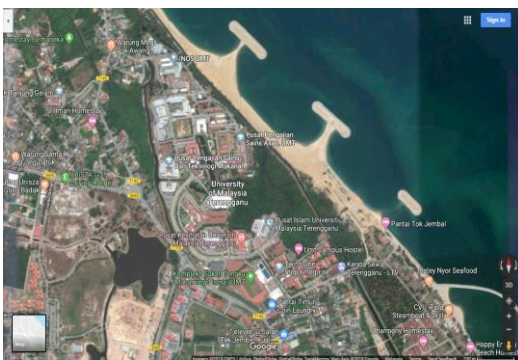


Figure 2: Two sampling sites in Universiti Malaysia Terengganu (UMT) campus. Right circle represents the location of *Bina Syarahan* building and the left circle is the location of the School of Marine and Environmental Sciences building

Observations of Flower Visitors

Observations to document the potential pollinators of the two species were conducted from August to September 2016 and January to February 2017. Direct observations were made by an observer from a distance of approximately 2m away from the flowering plants during the sunny days and equipped with a pair of binoculars (Minox BL 10x44 HD, MINOX, Germany). The diurnal observations were divided into three sessions, namely, morning (0700 hr to 1000 hr), afternoon (1100 hr to 1400 hr) and evening (1500 hr to 1800 hr).

For each hour, visitation frequency and foraging behaviour of the flower visitors were noted for only the first 10 minutes. A visit was considered when a flower visitor was at a close distance of the flower, (ca. 1-2cm) from the petals. Visits were recorded irrespective to previous visits by the flower visitors to the focal flower. The visitation frequency for each plant species was conducted for a total of 144 hours and 96 hours for *I. coccinae* and *R. simplex* respectively.

For the most common visitors to the flowers of each plant species, foraging activities of the flower visitors were further observed by focal sampling, and only recorded from 0700 hr to 1000 hr each morning for four days. The foraging behaviour of the flower visitors was recorded as either hovering or landing on flowers, and the time spent at the flower was recorded using digital stopwatch starting from when the visitor first approached the flower (ca 1-2cm distance from the flower) until it flew away. Insect samplings were further administered after the observations for identification using hand-held insect net. The insects were then identified by referring to the available insect collection from Herbarium and Insectarium of Central Laboratory, UMT. All statistical analyses were conducted in IBM SPSS Statistics v25.0 (Chicago, USA).

Results and Discussion

In this study, a total of 383 flower visitations by the insects (17 species) were recorded as compared to only a single species of bird, the olive-backed sunbird, *Cinnyris jugularis* (Table 1). From this, 276 visits to *I. coccinae* flowers were recorded and 107 visitations were made to *R. simplex* flowers. The most frequent visitor to the flowers was *Apis cerana* (Asian honey bee),

with 0.63 visits/hr and 1.00 visit/hr to *I. coccinae* and *R. simplex* respectively. For both plant species, the predominant visitors were insects, as compared to only one species of sunbird recorded visiting the flowers. *Ixora coccinae* was visited by a wide range of insect species, mainly the hymenopterans (eight species) and hemipterans (five species), as compared to only three insect species visiting *R. simplex*.

Table 1: List of flower visitors and their visitation frequency (visit/hr) to the flowers. Numbers in parentheses indicate number of visits (in %) recorded by each flower visitor observed in the first 10 minutes of every observation hour

Order	Famili	Species	<i>Ixora coccinae</i>	<i>Ruellia simplex</i>	
Hymenoptera	Apidae	<i>Apis cerana</i>	0.63 [@] (33.0)	1.00 ^{&} (87.9)	
		<i>Apis florea</i>	0.02 (1.1)	-	
		<i>Apis dorsata</i>	0.10 (5.4)	-	
		<i>Heterotrigona itama</i>	0.57 [@] (29.7)	-	
		<i>Xylocopa confusa</i>	0.26 [@] (13.4)	-	
		<i>Xylocopa violacea</i>	0.03 (1.8)	-	
	Vespidae	<i>Vespa tropica</i>	6.94 x 10 ⁻³ (0.4)	-	
		Unidentified sp. 1	0.04 (2.2)	-	
	Diptera		Unidentified sp. 2	1.39 x 10 ⁻² (0.8)	-
	Lepidoptera		Unidentified sp. 3	0.05 (2.5)	-
		Unidentified sp. 4	-	0.02 (1.9)	
		Unidentified sp. 5	-	0.02 (1.9)	
Hemiptera		Unidentified sp. 6	0.03 (1.5)	-	
		Unidentified sp. 7	0.04 (2.2)	-	
		Unidentified sp. 8	6.94 x 10 ⁻³ (0.4)	-	
		Unidentified sp. 9	6.94 x 10 ⁻³ (0.4)	-	
		Unidentified sp. 10	0.03 (1.5)	-	
Passeriformes*	Nectariniidae	<i>Cinnyris jugularis</i>	0.08 (4.0)	0.06 ^{&} (8.4)	

*Class Aves

[@]The three most frequent visitors to *Ixora coccinae*

[&]The two most frequent visitors to *Ruellia simplex*

Previous studies by Kato *et al.* (2008) and Duara (2014) reported a contrast in that lepidopterans from four different families; Papilionidae, Pieridae, Nymphalidae and Lycaenidae were the main flower visitors of *I. coccinae*. With their long proboscis, these butterflies could collect nectar from the long tubular *I. coccinae* flowers and therefore visited

the flowers to feed. Visits by the Asian honey bee (*A. cerana*) to *I. coccinae* flowers, however, might be for the pollen grains from the exposed position of the anthers which are located in between the corollas as hymenopterans are not equipped with suitable mouthpart to extract the nectar from the long small tubular flowers. Pollen grains are known to be consumed for

their protein content by the nectar feeding bats, *Syconycteris australis* (Law 1992a, 1992b).

Ruellia simplex was previously reported to be pollinated by the giant tropical bee, *Apis dorsata* which was also the first record of pollination by *A. dorsata* on *R. simplex* in Thailand (Hawkeswood & Sommung, 2016). The study stated that no other flower visitor, other than the giant bee, was present at the *R. simplex* flowers planted in a clump of about 100 plants, and therefore concluded that the giant bee was the only pollinator for the plant in that study area. Observation by Faria and Araujo (2010) on other species in Genus *Ruellia* (*Ruellia angustifolia*) reported that hummingbirds (the nectar-feeding birds) were the only visitors to the flowers. The long bills of these hummingbirds permit accessibility to the nectar of this long corolla flower and making regular visits to the flower increased their potential role as pollinators to the flower. Trip and Manos (2008) reported the transition between bee and bird pollinated species in Genus *Ruellia*, due to the lack of their main pollinator group which could lead to the shift in pollination mode of the plant species.

The hymenopteran and lepidopteran insects are among the most common flower visitors which contribute to pollination of the plants (Proctor & Yeo, 2009; Willmer, 2011; Rathnayake & Wijetunga, 2016). Pollination by these insect flower visitors particularly bees has long been highlighted due to their economic importance in crop production (Potts *et al.*, 2010; Nicholls & Altieri, 2013; Putra *et al.*, 2014). The long-tongued bees in the genera *Apis*, *Heterotrigona* and *Xylocopa* (Michener, 2000) are known to extract nectar from flowers with long corolla tube, collecting and transferring pollen grains while foraging on the flowers and therefore, act as pollinating agents to the flowers they visit. When compared to insects, many flower-visiting birds inflict more damage on the flowers rather

than contributing towards pollination (Willmer, 2011). Approximately 110 bird species from the family Nectariniidae (sunbirds and flower peckers) have been recorded as being involved in pollination, although not many details of their functions as pollinating agents can be found particularly in the Southeast Asian region.

Observations of foraging behaviour were conducted for the three main flower visitors of *I. coccinae* namely *A. cerana* (nine visits), *H. itama* (35 visits) and *Xylocopa confusa* (two visits) only (Figure 3). For *R. simplex*, only *A. cerana* and *C. jugularis* (Figure 4) were observed on 11 and 10 visits respectively. All the four insect visitors were found to land for foraging at the flowers. Landing on flower petals for feeding is a common foraging behaviour of insects as reported by Sarkar *et al.* (2014). In the study, they observed visitations to common ornamental plants (sunflower, *Petunia* and *Buganvillea*) which explained their role as pollen vectors and potential pollinating agents by contacting the flower parts. Although individual flower of *I. coccinae* is small, usually more than 10 flowers were densely packed in a rounded cluster of 5-13 cm (Duara, 2014) which allows the landing by these small insects on the flower cluster.



Figure 3: The landing feeding behaviour of the three main visitors of *Ixora* sp., A) *Apis cerana*, B) *Heterotrigona itama*, C) *Xylocopa confusa*

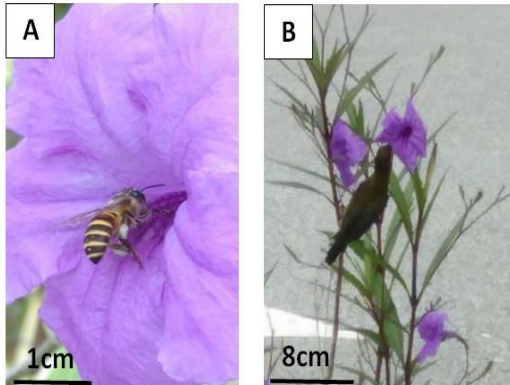


Figure 4: The two main visitors of *Ruellia simplex*. A) *Apis cerana* lands on the flower B) *Cinnerys jugularis* lands on the branch instead of the flower to collect nectar

For *R. simplex*, all the insect individuals were also observed to land on the flowers to feed, while *C. jugularis* were recorded to both hovering (20%) and landing (80%) for feeding at flowers. According to Pyke (1981), nectar-feeding birds employ hovering or landing mode

of feeding depending on the energy gained from the flower they feed on. As for *C. jugularis*, hovering while feeding might require more energy due to its large body size as compared to the energy gained from the nectar collected from *R. simplex* flowers, therefore landing is preferable. As compared to insect visitors however, this bird landed on the branch instead of flowers and collected nectar by inserting their long bill into the flowers.

For the time spent at the flowers (Table 2), *X. confusa* spent the longest time at *I. coccinae* with the mean (\pm SD) of 24.50 ± 20.51 sec, followed by *H. itama* (22.83 ± 23.33 sec) and *A. cerana* (10.11 ± 7.61 sec). However, the time spent was not significantly different between the three visitors (Kruskal-Wallis test, $H = 1.323$, $df = 2$, $p = 0.055$). For *R. simplex*, *A. cerana* recorded significantly longer time spent at the flowers (Mann-Whitney test, $U = 17.355$, $p < 0.001$) with 10.09 ± 4.04 sec. as compared to 2.90 ± 0.99 sec. recorded by *C. jugularis*.

Table 2: Time spent at the flowers (Mean \pm SD sec) of the main visitors of each flower species. Numbers in parentheses indicate range and n indicates the number of observations

Visitor species	<i>Ixora coccinae</i> *	<i>Ruellia simplex</i> **
<i>Apis cerana</i>	10.11 \pm 7.61 (2 – 28), n=9	10.09 \pm 4.04 (6 – 21), n=11
<i>Heterotriona itama</i>	22.83 \pm 23.33 (3 – 135), n=35	-
<i>Xylocopa confusa</i>	24.50 \pm 20.51 (10 – 39), n=2	-
<i>Cinnerys jugularis</i>	-	2.90 \pm 0.99 (2 – 5), n=10

*Not significant at $p = 0.05$

**Significant at $p = 0.001$

According to Silva et al. (2010), variation in time spent at the flowers is due to the availability of floral reward ie. nectar and pollen grains. Manetas and Petropoulou (2000) concluded that the amount of nectar could positively affect the duration of visits thus increase the pollination success of the flowers. This finding, however, disagrees with Putra et al. (2014) in which they reported larger sized honey bee (*A. cerana*) spent less time at the flowers of pepper

(*Capsicum annum*) as compared to stingless bee (*T. laeviceps*). Both bees showed an equal contribution to the pollination success to the flowers they visited, and they concluded that larger body size might compensate for their shorter visitation time. Larger sized pollinators, for example, carry larger pollen loads and can travel a long distance to promote cross-pollination (eg. Horner et al. 1998; Molina-Freaner et al. 2003).

Conclusion

According to their visitation frequency and foraging mode at the flowers, *A. cerana*, *H. itama* and *X. confusa* were the most important pollinating agents of *I. coccinae*. Not only did they showed high visitation rate, but these three insect species foraged by landing and spending more than 10 sec. to forage at the flowers. For *R. simplex* the most important pollinating agent was *A. Cerana*, which showed landing behaviour in every visit and spent a significantly longer time to forage at the flowers than the sunbird (*Cinnyris jugularis*). The sunbird, on the other hand, not only spent about 3 sec. at the flowers, but occasionally showed hovering feeding mode. Their vital role as pollinating agent to *R. simplex* nevertheless could be from their larger body size as compared to other smaller sized insect visitors to the flowers.

Acknowledgements

We would like to thank the School of Marine and Environmental Sciences for the equipment used and logistic support while conducting this research, Dr Norasmah Basari for her help in identification of the insects, and Prof. Dato' Dr. Mohd Tajuddin Abdullah for his constructive comments on this article.

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