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Pollinators and its behavior on *Delonix regia* (Boj. Ex Hook) Raf. (Family: Caesalpiniaceae)

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Abstract : The various parameters included in the study are Determination of flower life time and dynamics, Collection and identification of flower visitors, Observations on the behaviour of the foragers, Determination of forager mobility and duration of flower visits, Assessment of temporal depletion and deposition of pollen under flower –visitors activity at inflorescence level. The trees of *Delonix regia* bloomed after leaf shedding. It flowers during March – September. The period of blooming extended over 128.5 (R = 97 - 158) days. Individual flowers were very striking and prominent with one relatively larger, standard, upright petal marked with white, yellow and red streaks. It acted as a nectar guide and its base concealed the nectar. Its basal part formed a tube-like structure by the incurvation of margins. It functioned for 36 hours. The other four petals were scarlet-red. Flowers were retained for four days. Flowers were compatible for geitono and xeno pollination. Floral phenology with few flowers opening intermittently during the flowering life of inflorescences, staggered anther dehiscence and the visits of specialized pollinators, Papilionid butterflies - *Graphium agamemnon*, *Pachliopta hector*, *Papilio polytes*, *and Pachliopta aristolochiae*, Hawkmoth - *Macroglossum gyrans* promoted xenogamous pollinations. In day time open flowers sought by the carpenter bees (*Xylocopa Pubescens* and *X. latipes*) and honey bees (*Apis dorsata*) for pollen promotes geitonogamous pollination.
Keywords: *Diurnal Activity Of Foragers, Foragers Behaviour At Flower, Pollen Pick Up Efficiency, Visitors Foraging Speed.*

I. Introduction

Reproduction in flowering plants normally occurs by three ways. These include vegetative propagation, apomixis and sexual means. A plant can reproduce by any combination of these three ways. Sexual reproduction however differs from other means in that it results in the formation of new recombinant genotypes, thus giving way to the evolution of genetic diversity. This form of reproduction occurs in majority of plants suggesting that the formation of recombinant genotypes is of selective advantage (Stebbins.1950).

Flowers are the organs of sexual reproduction, and the sexual process involves two important events - Pollination and Fertilization, that lead to the formation of fruit and seed, i.e., the next generation and thus ensuring the continuity of species. The process of pollination involving an interaction with insects is believed to be basic to the evolutionary history of flowering plants. The evolution of more specialized interactions between plants and animals frequently transformed their inter- relationship from predation to mutualism in which both partners benefit; the animals are generally rewarded with food while the plant benefits in terms of both propagule dispersal (spores, pollen and /or seeds) and defense against herbivores (Pacini *et al.*, 2008).

Delonix regia (Boj. ex Hook.) Raf. (Syn. *Poinciana regia*) of the family Caesalpiniaceae is exotic - grown as an ornamental in India for its large red flowers. It is native to Madagascar. In India it has been successfully used in protecting channels and riverbanks. Keeping the above important aspects of biological conservation in view, study on pollination and its behaviour of *Delonix regia* of Caesalpiniaceae was undertaken. The study documents the pollination success of *D. regia* based on the flowering behaviour, insect-plant interaction and leading to pollination.

II. Materials And Methods

The present study on pollination ecology of *Delonix regia* (Boj. ex Hook.) Rafin. belonging to family *Caesalpiniaceae* was carried out during 2006 -2009. The study of *Delonix regia* present at different places of Visakhapatnam (17º41’N latitude and 82º18’E longitude) and Narasannapeta, Srikakulam district (18º20’N latitude and 83º50’E longitude), were utilized. These areas receive rainfall in the two monsoons – the North-East monsoon (December–February) and the South-West monsoon (June-September). The months of October and November receive the retreating monsoon rains and unpredictable cyclones. Temperature is highest during May, day temperature ranging between 30-40º C and night temperature between 20-28º C. Rainfall ranges between 100-150 cm per annum.

A brief description of the general distribution of study sites and economic importance of each species is given in the following. *Delonix* Rafin. is a genus of flowering plants in the family *Caesalpiniaceae.* It contains moderate sized trees native to Madagascar and East Africa. Among the different species of *Delonix* the best known species is *Delonix regia* (Boj. ex Hook.) Rafin. Its common names include gold mohur, peacock flower, royal poinciana etc. derived from its large flame red flowers. It is now wide spread in most tropical and sub-tropical areas of the world and is exotic in India. The plant mainly valued as a decorative tree, often being planted in avenues and gardens. It can also be planted as live fence posts and as shade tree in dairy forms, Tea plantations and compounds. The large pods as well as the wood are used as fuel. The tree yields gum and the seeds contain gum, that may be useful in textile and food industries. Bark has medicinal properties.

II.1collection Of Blooming Season Data And Floral Discription:

The period from the first opening of the flower buds to the time when the last flower completes its life was taken as the flowering period. After making cursorial observations of the flowering period of the study plant, field trips were undertaken at weekly intervals to different study sites to record the blooming period including the onset, progress and termination of the blooming of selected 25 trees for *D.regia*. The nature, morphology, orientation and arrangement of individual flowers on the inflorescence were carefully observed. Six different inflorescences each distributed on a different tree were tagged before the initiation of blooming. They were followed every day to record the number of opened flowers. The opened flowers were removed after counting to avoid their recounting the next day. These inflorescences were continuously observed until they ceased flowering to obtain data on their life span. The daily rate of flower production, and hence the flowering life of each inflorescence was thus assessed. The nature of inflorescence, the orientation of flowers, their morphology, petal colour and nectar production were recorded.

II.2. Collection And Identification Of Flower Visitors:

Representative specimens of the flower visitors at the study sites of the plant species were caught while they were foraging at the flowers, using insect catching net. The collected specimens were brought to site laboratory and identified by a comparison with the specimens already identified by CAB International Institute of Entomology, British Museum, London, U.K., and the Zoological Survey of India, available in our laboratory.

The relative importance of pollinator was assessed on the basis of general observations on the occurrence and abundance of flower visitors and intrafloral behaviour at different phases of flowering for each of the plant species. The flower visitors were censused on six clear sunny days during the flowering season. Attention was focused on a restricted part of flowering branch and each visitor species encountered was counted ten minutes in every hour.

II.3. Observations On The Behaviour Of The Foragers:

The flower visitors were observed on several occasions during daylight hours on each plant species for their behaviour i.e., mode of approach, landing, the type of forage they collected, contact with essential organs, etc. The foragers at work were photographed as far as possible using Olympus digital camera.

II.4. Determination Of Forager Mobility And Duration Of Flower Visits:

The forager at the flowers was followed on its track as long as the visitor was not away from clear view. The number of flowers foraged by the forager was counted and the total time spent to cover these flowers was recorded with a stop watch. From these data, the mean number of flowers visited in a unit time (minute) was calculated. The duration of the foraging visit was taken as the time from the movement the visitor alighted on the flower to the moment it left the flower. Ten such observations were made and the mean length of a visit in seconds was arrived.

II.5. Assessment Of Temporal Depletion And Deposition Of Pollen Under Flower –Visitors Activity:

The number of pollen per flower was determined at the time of anther dehiscence. As soon as the foraging activity of the flower visitor began, a fixed number of foraged flowers were removed at 4 h intervals, and the pollen remained in the collected anthers was counted. Subtracting this number from the previous number gave the number of pollen removed or depleted during the said interval. At the same time, stigmas were collected, pressed between two microscope slides, stained with cotton-blue, and examined for pollen under light microscope. The number of pollen deposited on the stigmas was then recorded. Ten such counts were made separately for pollen depletion from anther and pollen deposition on stigmas, from the counts thus obtained, the mean number of pollen grains depleted and that deposited at 4 h interval was assessed.

III. Results

III.1.Blooming Season And Floral Discription:

Delonix regia tree is deciduous. Blooming season was March to September. On average the flowering period of 25 trees studied at different sites was 128.5 days and the range was 97 – 158 days. The individual flowers are very striking and prominent with scarlet-red or flame-red coloured petals. Each flower has four spoon shaped spathulate spreading petals. One upright large petal (the standard petal) is marked with yellow, white and red markings. The five petals are imbricate in ascending order. The upright posterior standard petal also differs from other spreading petals in that it acts as a nectar guide. The flower is complete and zygomorphic. Stamens are 10, free, exerted. The gynoecium is superior with containing the ovules. The flower faces side wards. Both androecium and gynoecium are curved, and almost stand opposite to the standard petal. The flowers are nectariferous. It is concealed at the base of the odd petal. Total amount of nectar secreted in flower lifetime is 6 µl. The basal part of standard petal is relatively broader than the basal part of other petals and forms a tube like structure by the incurvature of the margins which remain overlapping. Any animal visitor seeking nectar at this flower is guided by the odd petal and its folded base. The flowers are retained for 4 days. The odd posterior standard petal remains functional for 36 hours.

III.2. Flower Visitor Dynamics (Plate – I & Ii)
Composition And Relative Abundance:

In all, 9 insect species, 3 of Hymenoptera, 6 of Lepidoptera were encountered at flowers during the observation period (Fig-1 & Fig-2). The Lepidoptera accounted 78.63% of total flower visits and followed by Hymenoptera 21.37%. Within the group of Lepidoptera *Macroglossum gyrans* was most dominant with a contribution of 18.27%, followed by *Pachliopta hector* 17.92%, *Graphium agamemnon* 17.5%, *Catopsilia pyranthe* 16.35% *Pachliopta aristolochiae* 15.4%, *Papilio polytes polytes* 14.56% of Lepidopterian visits. Within the Hymenoptera, *Apis dorsata* dominated and contributed of 58.75% total Hymenopteran visits followed by *Xylocopa latipes* 21.67% visits and *Xylocopa pubescens* 19.58% of total visits. Individually *Macroglossum gyrans* made the maximum number of total visits (14.35%), followed *Pachliopta hector* (14.13%), *Graphium agamemnon* (13.75%), *Catopsilia pyranthe* (12.85%), *Apis dorsata* (12.55%), *Pachliopta aristolochiae* (12.1%), *Papilio polytes* (11.44%), *Xylocopa latipes* (4.63%) and *Xylocopa pubescens* (4.2%) (Table-1).

III.3. Diurnal Activity Of Foragers:

The 9 insect visitor species were diurnal in their daily activity (Table-2). In the group of Hymenoptera, *Apis dorsata* appeared at 0700 hours and it was very brisk up to 0800-0900 hours. In the following hours it disappeared and reappeared at 1600 hours and was very brisk up to 1800 hours. It made cent percent visits during brisk time hours.

Other Hymenoptera insects *Xylocopa latipes* and *Xylocopa pubescens* appeared at 1100 hours increased up to 1200 hours and in the successive hours it began to disappear and terminated by 1500 hours. Both carpenter bees (*Xylocopa latipes* and *X. pubescens*) visit for pollen feed and the former made more visits than the latter. In the group of Lepidoptera, *Macroglossum gyrans* appeared at 0700 hours and it visits up to 0900 hours, it was disappeared in the successive hours and it reappeared at 1600 hours and more prominent up to evening (1900 h). It also made cent percent visits at brisk time. In the case of butterflies, they were very brisk at 0800 -1100 and 1500-1700 hours. *Graphium agamemnon* appeared at 0700 hours and made 97.2% visits at brisk time followed by *Catopsilia pyranthe* 97.3% and other butterflies *Pachliopta hector*, *Pachliopta aristolochiae* and *Papilio polytes* made cent percent visits at brisk time.

III.4. Visitors Foraging Speed:

Data on the number of flower visitors made in a minute and the time spent per flower by an insect species (Table-3) showed that among those visitors that frequented more, *Macroglossum gyrans* covered a large number of flowers in a minute time, followed by *Apis dorsata, Pachliopta hector, Papilio polytes, Graphium agamemnon, Pachliopta aristolochiae, Catopsilia pyranthe, Xylocopa pubescens* and *Xylocopa latipes.*

III.5. Foragers Behaviour At Flower:

Two groups of insects viz., Lepidoptera and Hymenoptera were observed to visit the flowers during daytime. Among the Lepidoptera, the papilionid butterflies, and the diurnal hawk moth were important. The other butterfly species found was *Catopsilia pyranthe*. Among the hymenoptera, the bees including the carpenter bees (*Xylocopa latipes* and *X. pubescens*), and honey bees (*Apis dorsata*,) appeared and collected pollen only. While collecting the pollen the bees settle on the stamen and collect pollen from the nearby anther of the flower. Their visits are therefore ineffective as they failed to contact the stigma. The papilionid butterflies including *Graphium agamemnon*, *Papilio polytes*, *Pachliopta hector* and *Pachliopta aristolochiae* visited the flowers for nectar. While taking nectar at the flowers they remained fluttering without alighting on the flowers. These foraging visits were evident at any time during day time but mostly they were numerous in the morning times. The fluttering wings and body always contacted the dehisced anthers and the stigma. Thus during the same visit both reception of pollen from anthers and deposition of pollen on stigma were possible. The hawkmoth *Macroglossum gyrans* is a fast mover, and was found to probe a large number of flowers within a short time. It also never settled at the flower. It hovered and collected the nectar, thus contacting the stigma, and anthers with the body and fluttering wings. Hawk moths were found more active in the morning and also in the evening. They were more prominent during the rainy season.

III.6. Pollen Pick Up Efficiency By Various Flower Visitors:

Washings of the visitor's body for the pollen grains indicated that *Pachliopta hector* pick-up more pollen grains (22.83%) than other visitors. It is followed by *Xylocopa pubescens* (17.62%), *Pachliopta aristolochiae (*15.85%), *Xylocopa latipes* (15.12%), *Papilio polytes* (14.12%), *Graphium agamemnon* (12.79%), *Catopsilia pyranthe* (8.80%) and least pollen pick-up is by *Apis dorsata.* (7.96%) (Table-4).

IV. Discussion

The results obtained and the observations made in respect of *Delonix regia* under study was discussed below in the light of available literature and established concepts of sexual systems and pollination ecology.

In other words, the idea of the most effective pollinator may have an overriding selective influence on floral morphology. Thus a harmony between flower morphology and colour, and the size and behaviour of pollinator is expected. In general, flower size is strongly correlated with the pollinator size and the ovule number is a function of the type of pollinator and its out-crossing ability. Accordingly, Proctor and Yeo (1972) and, Faegri and Pijl (1979) have catalogued and described the vast array of pollination systems and formulated pollination syndrome or packages of characteristics in plants serviced by different groups of pollinators. Variations in the floral traits directly involve with pollinator attraction and efficiency which can result in different reproductive outputs and further selection (Herrera, 1996). Facing of variable assemblage of floral displays, pollinators have shown to prefer plants with certain floral traits, such as high number of flowers (Mitchell et al., 2004; Benitez-Vieyra *et al*., 2006), large flowers (Ishi and Harde 2006; Celdon-Neghme *et al*., 2007), large nectar rewards (Thomson 1986; Fenster *et al*., 2006), inflorescence architecture (Crispin and Harder, 2006), or particular flowering time (Hall and Willis 2006). Flower colour and shape also influence pollinators (Kevan and Baker 1983; Waser 1983; Rodriguez-Girones and Santmaria 2004; Tastard *et al*., 2008; Whibley 2006, Altshuler 2003; Bradshaw and Schemske, 2003).

The peacock flower of *Delonix regia* fits butterfly syndrome or psychophily. The butterfly blossoms are characterized by diurnal anthesis with no closing at night, weak odour, petals vividly coloured including pure red, blossoms erect with flat rim, but often narrow with ample nectar held hidden in narrow tubes and nectar guide simple or mechanical tongue guide is simply a sexual groove. The sexual organs and nectar are well separated by long distance by the flowers of *Delonix regia* the anterior and lateral petals are bright scarlet-red and the posterior petal marked with yellow, white and red streaks at base forming a narrow tube by infolding of its margins (Sujan et.al. 2015). In *Delonix regia* flowers open during the day and continue in that condition the following night and day evening. The standard petal then becomes folded indicating that nectar is no more available for the pollinator. Strikingly, there is no flat rim because the petals are free and divergent. These flowers are characteristically probed for nectar by papilionidae butterflies, which normally do not alight on the flower; they remain hovering with their wings fluttering while taking the nectar. The wing span of these butterflies is large: *Graphium agamemnon* – 85 – 100mm; *Papilio polytes* – 90 – 100mm; *Pachliopta aristolochiae* – 80 – 110mm; *Pachliopta hector* – 90 - 110mm. According to Owen (1971), swallowtails regularly visit red flowers, which are not attractive to most other butterflies. *Delonix regia* flowers at the beginning of rains, and its abundant red flowers attract numerous swallowtails. Some individual swallowtails become so covered with red pollen from these flowers that in flight, they appear to be different exotic species and sometimes an individual is hardly able to fly because so much pollen is attached to the wings and body. While these butterflies forage at the flower, pollen grains get deposited on the underside of wings and the body and the same are transferred to the stigma during successive floral visits, thus performing pterygotribic or wing pollination. Cruden and Hermann- Parker (1979) reported butterfly pollination in *Caesalpinia pulcherrima*, the papilionid butterflies are being most important and effective pollinators in that species. The authors reported that pollen grains are held together by exinal connections, which results in the pollen having clumped dispersion pattern on the butterfly wing. They are seen in the photographs of Cruden and Jensen (1979). Since *D.regia* flowers have large number of ovules in the pistil, transfer of large number of pollen grains at a time would appear to be critical, or such transfer is aided by the clumping of pollen grains (Sujan et.al. 2015). Since the stigma area versus pollination wing area is small in wing pollination, small clumps of pollen scattered on the underside of the wings should increase also likelihood of pollination (Cruden and Jensen 1979). Apart from the butterflies, diurnal Hawkmoth *Macroglossum gyrans* visited the flowers for nectar. The moth visited a number of inflorescences foraging bout and perfectly handled *Delonix regia* flowers. Traits for butterfly pollination also attract Sphingids – tubular passage to conceded nectar (Wyatt 1983) and red colours strand out against green in crepuscular condition (Baker 1961). As such, the floral traits of *Delonix regia* may possibly be adapted for butterfly and moth pollinations. Though night observations were not made, noctuid moths may also have visited the flowers and accomplished pollination.

One of the possible reasons for short visitation sequences is the availability of nectar in such quantities adequate enough to attract and sustain the pollinator but not so much nectar that the pollinator becomes sedentary (Heinrich and Raven 1972, Heinrich 1975a). Though *Delonix regia* flowers are large the nectar quantity per flower is very small (0.1 – 0.6μl). Consequently, the lepidoptoran pollinators do not remain for longer periods on the same plant characteristically they visited few flowers per foraging bout. Hawkmoth visited comparatively more flowers than butterflies. Published data show that hawkmoths visit 2-7 flowers per plant and butterflies visit fewer than three flowers per plant.

Bhattacharya *et al.*, (1997) stated that butterflies used to visit *Delonix regia* and carry lot of pollen mass and thus help in dispersal. While the butterflies made visits throughout the flowering period of *Delonix regia,* diurnal hawkmoth was most prominent during the rainy season. Though the flowers are fully open by 1000 h, the receptive stigmas (1400 h onwards) and cent percent viable pollen (1530 h – 1830 h onwards) are available in the evening of Day 1, which time butterfly activity was at low ebb and hawkmoth activity at its peak. As such, the hawk moths assume greater importance as pollinators of *Delonix regia*.

Hawkmoth pollination appears to be more common in tropical habitat (Opler 1983). Hawkmoths are nectarivorous, strong and rapid fliers. In the biotope of the study area, the hawkmoth is the exclusive pollinator of *Xeromphis spinosa* (Subbareddi *et al.*, 1997), is an excellent forager on *Martynia annua* (Bhaskara Rao and Subbareddi 1994 a) and also on *Duranta repens* (Byragireddi and Subbareddi, 1996). Alexandersson and Johnson (2002) demonstrated appositive relationship between fitness (measured by the number of seeds produced) and naturally varying corolla tube length in the hawkmoth – pollinated Iris *Gladiolus longcollis*. Plants with shorter tubes were not effectively pollinated because of their mismatch with the tongue lengths of the majority of individuals of the hawkmoth pollinator *Agrius convolvuli*.

V.Acknowledgements :

This paper is dedicated to my beloved wife Pravallika and my son Mokshadith.

TABLE-1: Relative abundance of flower visitors and floral rewards of various anthophilous insects at *Delonix regia*

Visitor species	Total visit of six occasions	% of total visits	Floral resources sought	
			Pollen	**Nectar**
A. Graphium agamemnon	184	13.75		+
Pachliopta hector	189	14.13		+
Pachliopta aristolochiae	162	12.1		+
B. Papilio polytes	153	11.44		+
Catopsilia pyranthe	172	12.85		+
C. Macroglossum gyrans	192	14.35		+
D. Xylocopa latipes	62	4.63	+	
E. Xylocopa pubescens	56	4.2	+	
F. Apis dorsata	168	12.55	+	+

TABLE- 2: Diurnal activity of flower visitors on *Delonix regia* [The numbers of visitors are the means of six censuses]

Visitor species	700	800	900	1000	1100	1200	1300	1400	1500	1600	1700	1800	1900
Graphium agamemnon	1	2	7	12	6	0	0	0	3	5	0	0	0
Pachliopta hector	0	3	5	9	8	2	0	0	2	6	1	0	0
Pachliopta aristolochiae	0	2	4	5	3	0	0	0	0	3	0	0	0
Papilio polytes	0	2	4	5	2	0	0	0	0	3	1	0	0
Catopsilia pyranthe	0	3	6	8	7	0	0	0	2	5	6	1	0
Macroglossum gyrans	6	2	1	0	0	0	0	0	0	1	5	8	6
Xylocopa latipes	0	0	0	0	3	5	1	1	1	0	0	0	0
Xylocopa pubescens	0	0	0	0	2	4	1	1	0	0	0	0	0
Apis dorsata	3	5	4	0	0	0	0	0	0	4	5	2	0

TABLE-3: Foraging activity dynamics of various anthophilous insects at the flowers of *Delonix regia*

Visitor Species	No Of flowers visited					Length of visit			
	n	R	$\overline{X}$	S.D		n	R	$\overline{X}$	S.D
G. Graphium agamemnon	10	8-12	9.8	1.33		10	2-7	4.8	1.58
Pachliopta hector	10	9-16	12.5	2.22		10	3-7	5	1.34
Pachliopta aristolochiae	10	6-12	9.1	1.81		10	3-6	4.7	1.04
H. Papilio polytes	10	6-13	10.1	1.92		10	4-8	5.7	1.41
Catopsilia pyranthe	10	5 -11	7.6	2.00		10	5 -9	6.5	1.33

I. Macroglossum gyrans	10	18-36	25.4	5.54		10	1-3	2.2	0.17
J. Xylocopa latipes	10	5-9	6.9	1.44		10	5-16	10.3	3.24
K. Xylocopa pubescens	10	6-8	7.4	1.06		10	4-10	7.8	1.58
L. Apis dorsata	10	12-21	16.9	2.71		10	1-7	4.3	1.70

TABLE-4: Pollen pick-up by various flower visitors on *Delonix regia* as revealed by body washings

Visitors Species	**n**	**Number of pollen grains**		
		R	$\bar{X}$	S.D
M. Graphium agamemnon	5	41-258	157.8	78.08
Pachliopta hector	5	68-469	281.6	166.89
Pachliopta aristolochiae	5	82-345	195.6	107.11
N. Papilio polytes	5	56-278	174.2	74.98
Catopsilia pyranthe	5	36-189	108.6	55.35
O. Xylocopa latipes	5	78-327	186.6	91.08
P. Xylocopa pubescens	5	85-342	217.4	87.95
Q. Apis dorsata	5	34-147	98.2	38.13

n—No. of insects scanned
R—Range
$\bar{X}$ -- Mean no of pollen
S.D—Standard Deviation

PLATE - I

PLATE - II

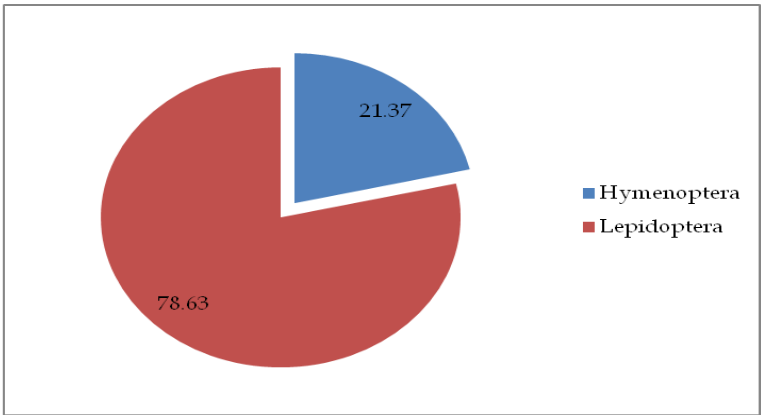

Figure: 1 : Visitor groups(%) on *Delonix regia*

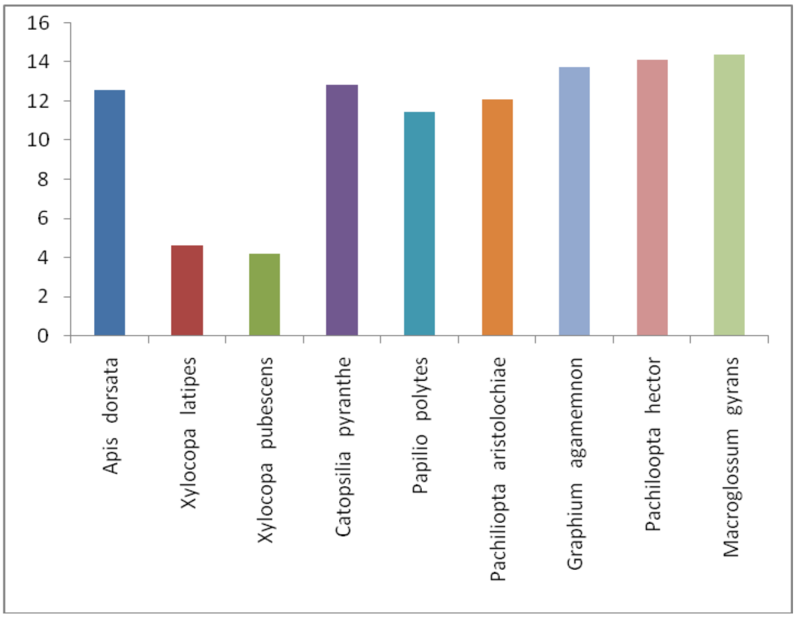

Figure: 2 : Individual visitor species(%) on *Delonix regia*

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