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THE POTENTIAL POLLINATORS OF *CAESALPINIA CRISTA* (LEGUMINOSAE : CAESALPINIOIDAE)

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ABSTRACT
Caesalpinia crista is one of the economically important medicinal plants of the family Leguminosae and is considered to be one of the best medicines to cure malaria. It is a large straggling, very thorny shrub with hooks and straight hard yellow prickles with nectaries at its base which attracts a considerable number of nectar feeding insects as it is an insect pollinated plant. The pollination ecology of *C. crista* was investigated through direct observation and video recording during the flowering period for three consecutive years (2009-2011) at Rani Reserve Forest, Assam, North Eastern India situated at 26º01' N to 26º06' N Latitude and 91º35' E to 91º42' E Longitude. In this present study, 22 species of insects were recorded in association with the plant among which 10 are major pollinators and the remaining 12 are minor pollinators of the plant. Among the 10 pollinator insects, seven potential pollinators was found to be exclusively bee species, *Apis cerana, Anthophora semperi, Xylocopa aestuens, Xylocopa latipes, Megachile disjuncta, Megachile latipes* and *Megachile sp.* Future conservation and management of this medicinally important plant species needs to take into account the needs and biology of its potential pollinators.

INTRODUCTION

Pollination and seed dispersal by animals are among the most important mutualistic interactions between plants and animals (Sekercioglu, 2006; Bascompte and Jordano, 2007; Corlett, 2007) and they contribute to crucial phases in the life cycle of plants (Jordano, *et al*., 2003; Aguilar *et al*., 2006; Sekercioglu, 2006; Kremen *et al*., 2007). Most plant species depend on animal pollinators for successful reproduction (Williams, 1996; Kremen *et al*., 2007; Michener, 2007).

Caesalpinia crista of the family Leguminosae is considered to be one of the best medicines to cure malaria. It is a large straggling, very thorny shrub with hooks and straight hard yellow prickles with nectaries at its base which attracts a considerable number of nectar feeding insects. It has entomophilous flowers capable of both self and cross-pollination. It is widely distributed from India to Polynesia. The pollination ecology of the *C. crista* was studied at China by Li *et al*. (2004). In India there is no record of the pollination biology and ecology of this plant which leads to the present study solely aimed to know the flower-visitor assemblages, flowering phenology, visitors' behavior, floral rewards and to identify the taxa that are likely to represent potential pollinators of the plant.

Keeping these points in view, a study was carried out in Assam on finding out the potential pollinators of *C. crista* in Rani Reserve Forest for better understanding of plant-pollinator interaction and to take up conservational efforts of this plant in the fragmented habitats of this highly disturbed area.

MATERIALS AND METHODS

Study Area

The study area, Rani Reserve Forest is situated at 26º01'N to 26º06' N L and 91º35' E to 91º42'E L with an altitude of 350 msl in Kamrup district, Assam, North Eastern India. It has an average annual rainfall of 2000cm and temperature range of 10ºC to 32ºC approximately. Rani Reserve forest is a mosaic of mixed deciduous, short grassland and semi evergreen forest covering an area of 4372. 380 Hectare. The landscape is hilly terrain with gentle slope. It is associated with wetland, Deeporbeel (Ramsar site) and paddy fields at its vicinity.

Climatic condition of Rani Reserve Forest, Assam

Ambient temperature, relative humidity and wind speed in Rani Reserve Forest for the three years (2009-2011) were collected from weather station (Airport, Guwahati, India). The average temperature ranges from 18.38ºC – 29.31ºC, relative humidity lies in between 69.9 - 84.85% and wind speed ranges from 1.15 – 5.85km/h.

Description of *Caesalpinia crista*

The plant is a prickly shrub or woody vine reaching a length of 10 meters or more. The leaves are bipinnate, often nearly 1 meter long, with the rachis armed with stout, sharp recurved spines. The pinnea usually number about 10 pairs and are about 20 centimeters long, with a pair of short, sharp spines at the point of attachment of each pair of leaflets. The leaflets also number about 10 pairs and are oblong, 2 to 5 centimeters in length and somewhat hairy. The flowers are yellow, borne in axillary, simple or panicled racemes and about 1 centimeter long. The pods are oblong, 5 to 7 centimeters in length, inflated and covered with slender spines and contain one or two seeds. The seeds are large, somewhat rounded or ovoid, hard, gray, and shining. Flowering started in the month of February. The flowers open in one day from 0730 to 0800h. The stigma is receptive to pollen for about 3 days after anthesis.

Pollinators' observation

All observations were made during flowering period. Pollinator abundance measurement was performed by following the method of Fenster (1991) by collecting all the hymenopteran pollinators but not during observation period. Observations on visitation rates were done by following the method of Kearns (1990). A branch of *C. crista* plant with receptive flowers was taken and marked with a tag. Morphological observations of floral form and variation were made in order to better inform observation during fieldwork. The number of individual flowers on the branch was counted and visits were recorded. The marked branch was observed for 10 minutes/observation, counting the number of visits of each insect species by following the capture-recapture method (Gary, 1971) for tagging bees. The plant was visited throughout the flowering period and observed at 0730-0830, 0900-1000, 1030-1130, 1200-1300, 1330-1430, 1500-1600 and 1630-1730h daily. Recorded insects were not captured. Insect frequencies recorded in the counts were used to analyze the effect of abiotic factors (independent variables: ambient temperature, relative humidity and wind speed) on the foraging activities and abundance of the pollinator insects. The activity patterns of the insects were also recorded. Other minor pollinators were also collected and recorded. Once the flower opened and the anther dehisced, pollen was collected, stained with fuchsin pink (Beattie, 1971), and mounted on a microscope slide. The resulting reference collection was used for identifying the pollen deposited onto the stigma by the insects. The pollen detected on the stigma of the flower after the first visit of each insect species was systematically dabbed with a 5 X 5mm square of fuchsin pink gelatin (Kearns and Inouye, 1993). These fuchsin gel pollen extractions were later melted onto glass slides and allowed to stand at least 24h for the stain to work, the pollen sample were later identified and counted under the stereoscopic microscope (100X magnification).

Statistical analysis

The collected data of foraging insects of *C. crista* flower and correlation coefficient between the abiotic factors and insect species at 5% significant level were analyzed with suitable statistical method through SPSS 16version

Finding out the potential pollinator

Visitor behaviour was estimated at five different behaviour decision levels in order to identify the potential pollinators of *Caesalpinia crista*.

Visitor activity

The purpose of visits for each insect species were categorized accordingly like- feeding on nectar (Fn), feeding on pollen (Fp), collecting pollen (Cp), nectar thieving (Theft) and nectar robbing (Rob). The first three contributes to pollination whereas the later two doesn't, so they were discarded.

Relative abundance

The number of flower visitors seen per branch daily. The high numbers were major visitors whereas the low numbers were minor visitors. Visitors with very low relative abundance were discarded.

Visitation rate

The total number of flower visits by each insect species per day per branch. High visitation rates contribute major pollination. Insects with very low visitation rates were discarded.

No. of flowers foraged

The number of flowers foraged by each insect species per minute. Higher the number higher the pollinator potentiality. Insects which foraged very less numbers of flowers were discarded.

Pollen detection percentage

The percentage of the number of stigmas on which pollen grains were detected after first visit of each insect species per virgin stigmas visited by them. Insects with very less pollen detection percentage were discarded.

RESULTS AND DISCUSSION

Phenology of *Caesalpinia crista*

Flower blooming time of *Caesalpinia crista* was from 0700 to 0800 hrs and no reference is available regarding this. Flowering started from February and lasted till March. All the fully developed buds of an inflorescence take a minimum of 8 days to open. However, Li *et al.* (2004) reported starting of flowering from March to May and he also reported opening of inflorescence from fully developed buds taking 10 days which is longer than the present finding. The reason may be due to the difference in environmental conditions between India and China. Full bloom flowers persist for 4-5 days at the most as similarly reported by Li *et al.* (2004). Fruiting takes about 4-5 months for the seeds to mature unlike the finding of Li *et al.* (2004) of more than five months. Again the reason may be due to environmental conditions.

Pollinator composition

A total of 22 pollinators were recorded during the study. Total visits made by the insects per daily counts recorded was 429.18. Pollinators in the present study were found to be more diverse than the findings Li *et al.* (2004) where he reported 17 species of insects from four orders. 14 species out of 22 pollinators were from the order hymenoptera from which it can be said that hymenopteran pollinators were the most common visitors and they were also the main pollinators. Similar result was reported by Li *et al.* (2004) in the same species and Sjodin (2006) in two semi-natural grassland communities. In case of the hymenopteran species, it was found that wild bees were more abundant in the pollinator community and the only honey bee present in the community was *Apis cerana*. Therefore, it can be said that wild bees are the major pollinators of the plant. and similarly, Li *et al.* (2004) also found only two honey bees *Apis cerana* and *Apis* sp.

Table 1: Foraging behaviour decision levels of insect sp. on *Caesalpinia crista*

Sl. No.	Insect taxa	Decision level 1	Decision level 2	Decision level 3	Decision level 4	Decision level 5	Potent Pollinator
		Visitor activity	Relative abundance	Visitation rate	No. of flowers foraged/min	Pollen detection (%age)	
1	*Apis cerana*	Fn,Fp	8.11±0.54	39.08±0.51	12.08±0.45	75	√
2	*Andrena certii*	Fn,Fp	2.91±0.20	31.8±0.13	6.5±0.27	35	
3	*Anthophora semperi*	Fn,Fp	3.96±0.27	38.5±0.57	9.56±0.13	90	√
4	*Tetragonula sp.*	Fn	2.10±0.15	20.77±0.36	5.54±0.28	0	
5	Chalcid sp.	Fn	1.16±0.08	28.54±0.26	7.04±0.56	0	
6	*Halictus sp.*	Fn,Fp	3.29±0.24	5.25±0.12	3.01±0.27	85	
7	*Megachile carbonaria*	Fn,Fp	4.25±0.27	16.98±0.38	4.98±0.33	38	
8	*Megachile disjuncta*	Fn,Fp	6.13±0.38	22.32±0.54	7.08±0.45	52	√
9	*Magachile lanata*	Fn,Fp	4.95±0.36	27.89±0.55	6.55±0.28	48	√
10	*Megachile sp.*	Fn,Fp	4.43±0.34	26.98±0.44	7.9±0.54	58	√
11	*Osmia sp.*	Fn, Fp	4.11±0.28	13.98±0.33	4.67±0.54	22	
12	*Vespa sp.*	Fn,Fp	1.07±0.07	9.31±0.08	0.77±0.2	20	
13	*Xylocopa aestuens*	Fn	2.2±0.09	35.78±0.13	9.45±0.09	100	√
14	*Xylocopa latipes*	Fn	2.12±0.07	32.09±0.45	8.56±0.07	94	√
15	*Episyrphus balteata*	Fp	3.47±0.18	17.9±0.40	2.37±0.58	15	
16	*Syrphus sp.*	Fp	2.08±0.12	12.39±0.07	1.83±0.27	14	
17	*Musca sp.*	Fn	1.79±0.13	8.33±0.20	0.36±0.07	0	
18	*Catopsilla pomona*	Fn	6.37±0.27	7.89±0.09	2.08±0.34	17	
19	*Catopsilla pyranthe*	Fn	6.07±0.27	12.69±0.50	3.08±0.5	29	
20	*Eurema andersonii*	Fn	3.61±0.40	6.19±0.54	1.92±0.56	5	
21	*Euploea core*	Fn	4.84±0.33	9.78±0.24	0.69±0.31	7	
22	*Terias hecabe*	Fn	1.33±0.13	4.74±0.07	0.78±0.09	0	

Fn = Feeding on Nectar, Fp = Feeding on Pollen; Visitation rate = No. of foraging visits/day; Pollen detection (%) = No. of stigmas on which pollen grains detected after first visit / No. of virgin stigmas visited X 100); " = Potential Pollinator.

Table 2: Potential pollinators - corresponding abiotic factors correlation during 2009-2011

Insect species	Abiotic factors Temperature (ºC)	Relative humidity (%)	Wind speed (km/h)
Apis cerana	-0.53**	-0.1	-0.5**
Anthophora semperi	-0.66**	-0.11	-0.37**
Megachile disjuncta	-0.56**	-0.09	-0.45**
Megachile lanata	-0.6**	-0.13	-0.41**
Megachile sp.	-0.6**	-0.17	-0.36**
Xylocopa aestuens	-0.51**	-0.17	-0.28**
Xylocopa latipes	-0.57**	-0.12	-0.4**

* Significant at 5 %

Pollinator behavior

Most insects were found to have a clear peak of activity in the 1000h in the morning and 1500h in the afternoon and are very scarce after 1700h. Similar result was reported by Vicens and Bosch (2000) in case of *Apis mellifera* and other insects. Ten visitors were observed feeding on nectar and pollen, another ten feeding on nectar and only two visitors were observed feeding only on pollen (Table 1). Similarly, Li *et al.* (2004) reported nine species feeding on nectar alone, however the nectar and pollen combination feeders were amounted to only three species and only four pollen visitors were reported. Other insects like ants, beetles and other miscellaneous insects which visit the plant for various purposes like nesting, feeding on nectaries, robbing nectar and predating upon the visitors were discarded due to their non participation in pollination of the plant. Differences in visitor activity (Decision level 1) were best explained by types of resources available and differences in visitor activity diversity were also related to flower abundance as reported by Sjodin (2007).

Among all the pollinators the abundance of *Apis cerana* (8.11±0.54) was the highest, followed by *Catopsila Pomona* (6.37±0.27) and *Megachile disjuncta* (6.13±0.38) while that of the Chalcid sp., *Vespa* sp., *Musca* sp., and *Terias hecabe* were the lowest (Table 1). Differences in the relative abundance of the pollinators (Decision level 2) were based on the differences in the number of flowers in bloom. The number of flowers may reflect the amount of reward for visitors and visitors seemed to be good at estimating the amount of resources on this scale (Dreisig, 1995). The visitation rate of *A. cerana* was found to be highest (39.08±0.51) followed by *Anthophora semperi* (38.5±0.57), (Decision level 3) (Table 1) unlike the findings of Li *et al.* (2004) in which *Xylocopa sinensis* and *Xylocopa* sp. were with the highest visitation rate. Lepidopteran species *T. hecabe* (4.74±0.07) was observed the lowest (Table 1). Li *et al.*, in 2004 also reported lowest visitation rate in lepidopteran species.

A. cerana, A. Semperi, X. aestuens, X. Latipes were the highest in number of flowers foraged per minute (12.08±0.45, 9.56±0.13, 9.45±0.09, 8.56±0.07 respectively) (Decision level 4, Table 1) contributing to cross pollinations of many flowers in a minute. But this decision level alone cannot be ruled out for finding the efficiency of the pollinators without considering the pollen detection percentage which held the key to the success of the pollination. In the present study, five decision levels were tested in order to find out the potential pollinators of *Caesalpinia crista* including the pollen detection percentage on stigmas after first visit. However, Freitas *et al.* (2002) found out potential pollinators from an array of flower visitors of Cashew pollination in NE Brazil by using the Spear's (1983) index of 'single-visit pollinator efficiency'.

Highest pollen detection percentage was recorded in *X. aestuens* (100%) followed by *X. latipes* (94%), *A. semperi*

(90%) bringing maximum number of pollen to the stigmas in a single visit (Decision level 5) while *T. hecabe*, *Tetragonula* sp. and Chalchid sp. and *Musca* sp. were lowest with 0% each (Table 1). However, Li *et al.* (2004) found Xylocopa sp. with the highest pollen detection percentage.

Potential pollinators

Seven visitors of the hymenoptera order viz., *Apis cerana, Anthophora semperi, Megachile disjuncta, Megachile lanata, Megachile sp., Xylocopa aestuens, Xylocopa latipes* were identified as the potential pollinators of *Caesalpinia crista* after proving positive results in all the five behaviour decision level tests (Table 1). Although *M. disjuncta, M. lanata* and *Megachile* sp. were not detected with very high pollen detection percentage but with average percentage (52%, 48% and 58% respectively) (Decision level 5), they were also regarded as potent pollinators when overall decision levels were considered (Table 1). Li *et al.* (2004) reported *Xylocopa sinensis, Xylocopa* sp., *Apis cerana, Episyrphus balteata* and *Syrphus* sp. as the potential pollinators of the plant and *Xylocopa* sp. as the most important pollinator. The differences in the result may be due to the differences in the pollinator composition between his findings in China and the present findings in India. In case of *Halictus* sp. eventhough its pollen detection level was very high (85%), it was not regarded as potent pollinator due to its very low relative abundance (3.29 ± 0.24), low visitation rate (5.25 ± 0.12), and low number of flowers foraged per minute (3.01 ± 0.27) (Table 1). Ali *et al.* (2011) also found that *Halictus* sp. had the highest pollen deposition in *Brassica napus*, but it was not regarded as the best pollinator due to its very low visitation rate.

Overall *X. aestuens* was found to be the most important pollinator as similarly observed by Li *et al.* (2004) due to their body size and structure they fit perfectly into the flowers bringing 100% pollen detection in a single visit. Following *X. aestuens* are *A. semperi, X. latipes, A. cerana, M. sp., M. disjuncta and M. lanata* respectively with as much pollinating potential without them *C. crista* plants would be in shrinking population.

Potential Pollinators - Abiotic factors correlation

All the potential pollinators were found to be negatively and significantly correlated with temperature. No significant correlations were observed with relative humidity. All the potential pollinators were also found to be negatively and significantly correlated with wind speed at 0.01% significance level (Table 2). All pollinators showed activities at relative humidity below 90%. Most of the pollinators were not recorded at temperature above 29°C except *Xylocopa aestuens and Xylocopa latipes* which were seen even at temperature upto 30ºC. They were also observed at wind speeds upto 8 km/h. Eventhough abiotic conditions, especially temperature seemed to be affecting the abundance and visitation rates of the pollinators, the indifference in the case of *X. aestuens and X. latipes* may be because of their sturdiness and large and heavy body weight that can withstand these inhibiting factors. Li *et al.* (2004) however, has not reported the effects of environmental conditions in the pollinator community of *Caesalpinia crista.*

The present study highlights the value of studying behaviour in a conservation context. It is very important to find out the potential pollinators of a particular plant of importance from an array of flower visitors for the maintenance and practice of successful propagation and production for their appropriate management. Therefore, for the fruitful pollination and successful propagation of this plant, these seven potential pollinators need to be taken with serious attention. The future conservation and management of this medicinally important plant species needs to take into account the needs and biology of these potential pollinators.

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