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Detailed floral biology and flower visitors of Karanja, *Pongamia pinnata* (L.) in Karnataka, India

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Abstract
The *Pongamia pinnata* is being an important biofuel plant which is distributed in the tropical and subtropical areas of the world, pod yield of this plant is very much negligible due to scarcity of information on the floral biology and the flower visitors of *P. pinnata*. The present study was undertaken at the UAS, GKVK, Bengaluru to document its floral biology and the flower visitors in the South Eastern Dry Zone of Karnataka. Flower structure, anthesis, anther dehiscence and pollen to ovule ratio, pollen germination, stigma receptivity and flower visitors were documented and studied in detail. The results revealed that the flowering (acropetal) period was observed between first fortnight of march to first week of May month with a peak period of 20-25 days. It has a unique floral architecture with bisexual, zygomorphic and have one large standard petal, with two light-purple wing petals and two white keel petals. Anthers and stigma are enclosed but separated in the boat-shaped keel petals. Anthesis was observed at 0830 h and reached peak at 0900 h and started decreased at 1000 h. Anther dehiscence was noticed 1 or 2 hours prior to anthesis. Pollen to ovule ratio was 15125±1375.63: 1. The Pollen germination was 92.28 to 94.21% and stigma receptivity was 77.03 to 85.82% was maximum between 1000 to 1200 h. During the study the major flower visitors were Megachilid and Honey bees followed by Thrips and Syrphids.

Keywords: Karanja, floral biology, anthesis, germination, bees, ovule stigma receptivity

Introduction
The *Pongamia pinnata* (L.) is often known by the synonym *Millettia pinnata* and it is native to India (Sujatha *et al*., 2008) [1]. *Pongamia* is a genus consists of about 150 species, which are distributed in the tropical and subtropical regions of the world (Scott *et al*., 2008) [2]. *P. pinnata* (L.) is commonly known as Karanja, Indian-beech, Poonga-oil-tree, Pongam tree, Karum and Kanji in India. The species thrives in areas with an average rainfall of 500-2500 mm and a temperature range of 1-38 °C. It can withstand drought and waterlogging and a slight frost. It can be grown in water logged, saline and alkaline soil, waste land and can resist harsh agro climates. It grows about 15-20 meters in height with a wide canopy that spreads equally wide. Flowering starts after 4-5 years of planting and the productivity per hectare varies from 900 to 9000 Kg per hectare. The seeds contain about 30-40 per cent oil. The major fatty acids in crude oil of *P. pinnata* are palmic acid, stearic acid, linoleic acid and ecosenoic acid, these fatty acids exhibited good physio-chemical properties and could be used as a biodiesel feedstock and industrial application (Bobade and Khyade, 2012) [3]. Other than biodiesel, it can also be a source of raw material for the bio-pesticides, biologically dried leaves are being used as an insect repellent in stored grains. The press cake, when applied to the soil, it has a pesticidal value, particularly against nematodes and conventional medicines (Danial, 1997) [4]. This species has a lot of potentials to be utilised as biofuel plant but the major limiting factor is scattered knowledge available on floral biology, flower visitors and pollination of Karanja (Kaushik *et al*., 2021) [5]. Hence, the aim of this study is to document the floral biology and flower visitors of *P. pinnata* which in turn helps to overcome the yield constraints.

Material and Methods
The present investigation was carried out at the University of Agricultural Sciences, Gandhi Krishi Vignana Kendra (GKVK), Bangalore located in North part of Bangalore in the south eastern dry zone of Karnataka state. The observations were recorded from Karanja plants with six year old trees.

Flower structure
Fully opened flowers were brought to the laboratory and examined for their structure under microscope. The floret parts such as sepals, petals, stamens, anther lobes were measured (n = 10) using a stereo binocular microscope (Leica M205C with auto montage and Leica DFC 450 camera) (Revanasidda and Belavadi, 2019 [6], Veereshkumar *et al*., 2019) [7].

Anthesis, anther dehiscence and pollen to ovule ratio
The time of flower opening was observed and recorded by tagging a set of flower buds (n=90). The tagged buds were observed at a half an hour interval from 0500 h to until all the flowers were opened. A set of flower buds were tagged (n=90) and observed for anther dehiscence at different times of the day from 2h before anthesis by using hand lens. After anther dehiscence, flowers (n=10) were brought to the laboratory and counted the total number of pollen grains by extracting all pollen into glacial acetic acid in 1 ml plastic vials and counted using haemocytometers (Kearns and Inouye, 1993) [8]. The number of ovules per ovary was recorded by longitudinally dissecting the ovary (Kaushik *et al*., 2021) [5].

Pollen germination
Pollen germination test was by collecting fresh flowers at two hour regular intervals from 2h before anthesis to 8h after anthesis and brought to the laboratory. In *vitro* study was done by transferring pollen onto separate cavity slides containing 10% sucrose solution, Ca $(NO_3)_2$ (164.08mg), H_3BO_3 (61.83 mg) and $MgSO_4$ (120.36 mg). After 24 h, pollen germination was recorded to work out the peak pollen germination period (n=10) (methods adopted from Belavadi and Ganeshaiah, 2013 [9]; Revanasidda and Belavadi, 2019 [6]).

Stigma receptivity
To study the stigma receptivity, a set of flowers were emasculated at 0600 h and were bagged to prevent the flower visitors. For every two-hour interval, one set (n=10) was hand pollinated. After 24 h, pollinated stigma was preserved in Carnoy's fixative (absolute ethanol: chloroform: acetic acid; 6:3:1). Pistils were softened with 16 M NaOH for overnight. Pistils were stained with aniline blue (1%). Samples were viewed through a Leica DM2000 (HI PLAN 100X/1.25 DIL) with a fluorescent filter I3. Per cent of germinated pollen grain was scored as receptive (Huang *et al*., 2004) [10].

Flower visitors
All insects visiting flowers of Karanja were recorded by following methods: Visual counting and Ad-libitum visual counting of flower visitors were recorded from 1000 h to 1200 h on a given sampling day with a recording time of fifteen minutes and a time gap of five minutes between two observations. (As per the method adopted by Revanasidda and Belavadi, 2019 [6]; Veereshkumar *et al*., 2019 [7]).

Results and Discussion
The flowering period of *P. pinnata* observed in between first fortnight of march to first week of May, some of individual plants extended their blooming up to first week of June. The total flowering period took almost one month, with 20-25 peak flowering days. Flowers remained open only for a single day in *P. pinnata*. The flowers begin to senescence slowly from 1700 h onwards on the day of opening and close completely at 1800 h. During senescence, standard petal come in contact with the wing and keel petals completely indicate closure of the flower.

Flower structure
Pongamia flowers are bisexual, purplish white in color. The anthesis within an inflorescence followed acropetal opening over a period of 8.58±1.16 (Mean±SD) days. Individual flowers measured 15.90±0.21 X 7.42±0.15mm. The standard petal was large (15.96±0.10X17.50±0.21mm), light purple, with a patch of charter use green at 1/3rd above the base. The two wing petals were purplish white and streaks on the petals which measured about 14.43±0.14 X 5.34±0.24 mm. Two keel petals are boat-shaped and measured 12.78±0.11 X4.44±0.24 mm. Unfolding of the standard petal indicates flower opening. The calyx of the flowers is cup-shaped, truncate and dark brown in color. The corolla is white to pink and brownish-veined on the outside. There are ten stamens, each filament (10.64±0.17 mm long) bearing small anther lobes (0.77±0.03 mm long and 0.39±0.03 mm wide). Stamens are diadelphous; with nine stamens united into one bundle and the tenth one in free condition. The bundled stamens form staminal tube at the base and become free towards the apex. In comparison, the style was longer (13.61±0.41 mm long and 0.86±0.13 mm wide) bearing stigma lobe (0.32±0.02 mm long and 0.27±0.02 mm wide). Flower description was presented in Table 1 and Fig.1.

Table 1: Morphometric features of *P. pinnata* flower (n=10)

Features	Mean±SD
Pre-anthesis bud (L)	9.90±0.27
Pre-anthesis bud (W)	3.71±0.10
Flower (L)	15.90±0.21
Flower (W)	7.42±0.15
Style (L)	13.61±0.41
Style (W)	0.86±0.13
Stigmatic lobe (L)	0.32±0.02
Stigmatic lobe (W)	0.27±0.02
Anther 10th filament (L)	10.64±0.17
Length up to 9 filaments united	10.08±0.10
Anther lobe (L)	0.77±0.03
Anther lobe (W)	0.39±0.03
Standard Petal (L)	15.96±0.10
Standard Petal (W)	17.50±0.21
Wing Petal (L)	14.43±0.14
Wing Petal (W)	5.34±0.24
Keel Petal (L)	12.78±0.11
Keel Petal (W)	4.44±0.24
No. of anther/flower	10±0.00
Flower width at base	6.72±0.21

Note: L-Length; W-Width

Anthesis, anther dehiscence and pollen to ovule ratio
The anthesis began in the morning 0830 h (16.66%) and peak flower opening was seen at 0900 h (47.44%) and it was reduced at 1000 h (11.11%) and there was no flower opening after 1030 h. There were 9 to 10 flowers opened per inflorescence per day. All flowers were opened in an inflorescence within 8 to 10 days (Fig. 2). All the ten anthers dehisced by longitudinal slits in mature bud stage, 1 or 2 h prior to anthesis. Anther dehiscence started at 0630 h (11.11%) and reached peak at 0700 h (37.77%) and then started decreasing after 0830 h and there was no anther dehiscing after 0930 h.

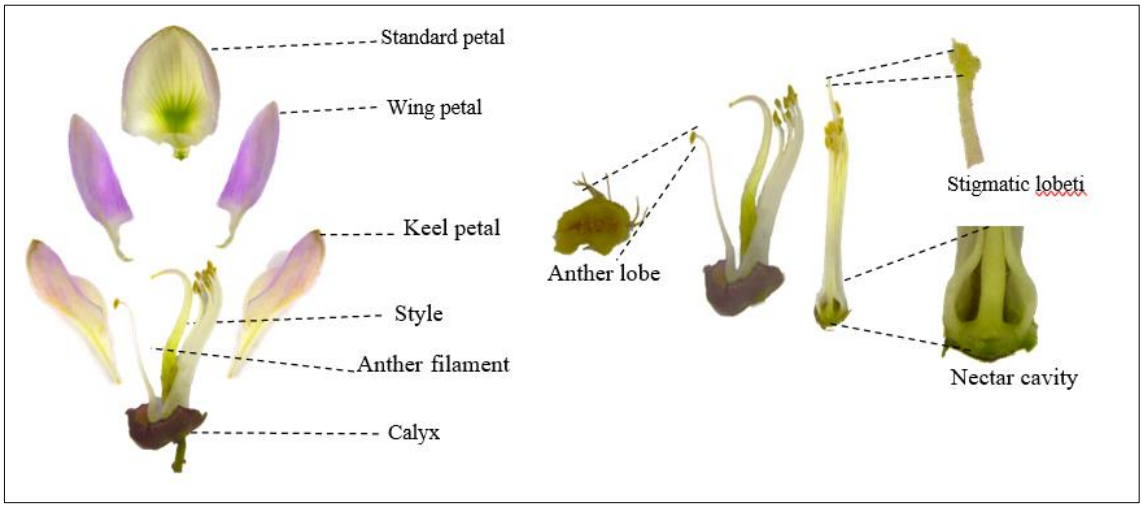

Fig 1: Floral structure of *P. pinnata*

Pollen germination

The number of pollen grains present per flower ranged from 25000 to 32500 with a mean of 30250.00±2750.12 and the number of ovules per flower was two. Pollen grains per ovule of flower ranged between 12500 to 16250 with a mean of 15125±1375.63. Pollen to ovule ratio was found to be 15125±1375.63: 1. Pollen germination was observed in flowers of Karanja from 0600 to 1800 h (Fig. 3). Pollen germination was started raising from 0800 h (35.06%) and peak germination was recorded from 1000 to 1200 h (92.28 to 94.21%) and started decreasing from 1400 h and very low germination per cent recorded at 1800 h (5.78%) (Fig. 3).

Stigma receptivity

Stigma receptivity was recorded from 0800 h to 1800 h. The peak stigma receptivity was observed between 1000 to 1200 h (77.03 to 85.82%), after started decreasing. There was no stigma receptivity after 1600 h (Fig. 3).

Flower visitors

Flower visitors recorded were mainly belonging to order Hymenoptera, Thysanoptera and Lepidoptera group. Megachilid bees (11.4 bees/ 15 min) and Honey bees were the major flower visitors as compared to other groups. However, thrips and syrphids were also visited plants frequently next to Bees (Table. 2).

Pongamia pinnata is a species of family Leguminosae, which has unique floral architecture. Bisexual, zygomorphic flowers have one large standard petal, two light-purple wing petals and two white keel petals. Anthers and stigma are enclosed but separated in the boat-shaped keel petals (Aronne *et al*., 2012) [11]. The *Pongamia* initiated flower buds in the month of First week of April. The flowering period was between first fortnight of march to first week of May month. The present findings are corroborated with the previous reports mentioned that *P. pinnata* initiated flower buds from mid-April to mid of May in Tamil Nadu (Srimathi *et al*., 2013) [12], the first week of March and May in Maharashtra (Kukade and Tidke, 2013) [13], April to the first week of June in Andhra Pradesh, first week of May and lasted till the first week of June in Uttar Pradesh (Kaushik *et al*., 2021) [5]. The inflorescence of *P. pinnata* is a long raceme, anthesis occur acropetally over a period of 11±4 days (Raju & Rao, 2006) [14]. The flowers begin to senescence slowly from 1700 h onwards on the day of opening and close completely at 1800 h. The results are in line with the observations made by Raju and Rao (2006) [14]. The anthesis began in the morning 0830 h and peak flower opening was seen at 0900 h. All flowers were opened in an inflorescence within 8 to 10 days. Similar observations were made by Raj and Rao (2006) [14], Kukade and Tidke (2013) [13], Ni Luh *et al*. (2014) [15], who have reported the time of anthesis during 0700 to 0930 h.

Table 2: List of flower visitors recorded on *P. pinnata* flowers in GKVK, Bengaluru

Pollinator	Family	Order	Mean visitors per 15 min.	
Megachilidae bees	Megachilidae	Hymenoptera	11.44397	
Apis florea	Apidae		7.444117	
Apis cerana			7.193625	
Apis mellifera			5.094129	
Apis dorsata			5.255373	
Thrips	Thripidae	Thysanoptera	5.094129	
Yellow jacket wasp	Vespidae	Hymenoptera	3.863703	
House fly	Muscidae	Diptera	4.381341	
Syrphid fly	Syrphidae		5.094129	
Skippers	Hesperiidae	Lepidoptera	3.346065	
Lycanids	Lycanidae		3.863703	
Monarch butterfly	Nymphalidae		3.346065	
			SEm	0.1500
			CD @ 5%	0.4302

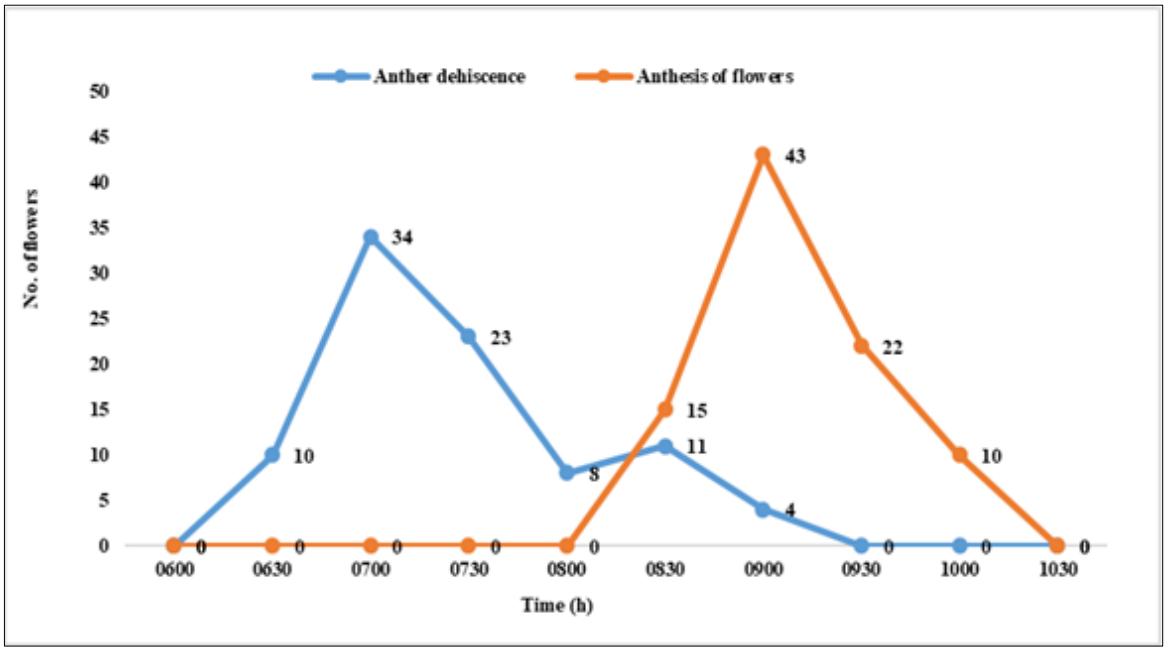

Fig 2: Time comparison between anthesis and anther dehiscence in *P. pinnata*

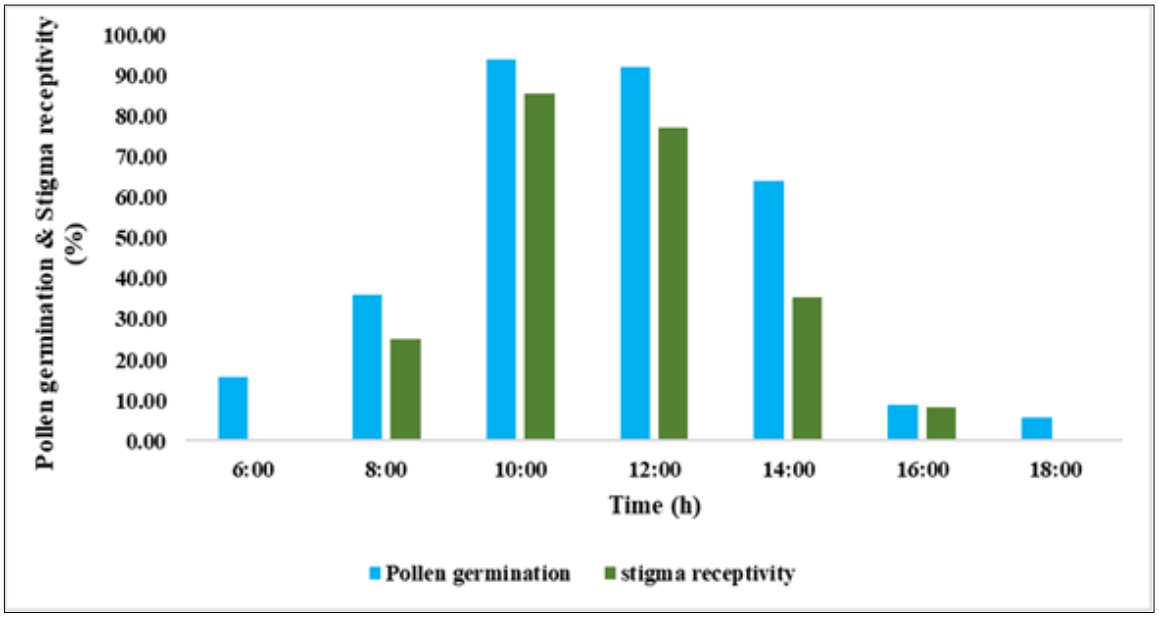

Fig 3: Comparison of Pollen germination and stigma receptivity in *P. pinnata*

All the ten anthers dehisced by longitudinal slits in mature bud stage, 1 or 2 h prior to anthesis. Raj and Rao (2006) [14], Kukade and Tidke (2013) [13], Ni Luh *et al.* (2014) [15] have reported that all the ten anthers dehiscing approximately 3 h before anthesis in Karanja. Pollen to ovule ratio was found to be 15125±1375.63: 1. Similar observation was made by Arathi *et al.* (1999) reported that the *P. Pinnata* ovary contained two ovules (95.04% of the flowers) and occasionally three (4.96%), with a mean of 2.05±0.22 ovules per ovary [16].

Pollen germination was observed in flowers of Karanja from 0600 to 1800 h. Kukade and Tidke (2013) reported that pollen viability percentage in *P. pinnata* with TTC was found to be 82.98%, whereas, it was found to be 41.45, 28.45, 54.38, 21.84 and 15.89% with different concentration of sucrose solution *i.e.*, 10, 20, 30, 40, 50%, respectively [13]. In *vivo* pollen germination, the per cent of pollen germination was found to be 47.91% on the day of flower opening and 56.25 and 50% on the next two days. Stigma receptivity was recorded from 0800 h to 1800 h. The peak stigma receptivity was observed between 1000 to 1200 h. The results are in conformity with the observations made by Raj and Rao (2006), they reported that stigma achieves receptivity one hour after anther dehiscence, peak receptivity was between 0900-1600 h in *P. pinnata* [14].

The major flower visitors are belonging to Megachilidae and Apidae of order Hymenoptera. Present findings were in line with earlier reports where Megachilid bees were found to be the most abundant and contributing for pollination in Karanja flowers (Kaushik *et al*., 2021) [5]. Further, Dhillon *et al*., 2009 also observed that bees and thrips are the major flower visitors of Karanja and they contribute significantly in pollination [17].

Conclusion

Pongamia pinnata is one of the important bio-fuel tree

species in India. The floral biology of Karanja plants is important for determining yield constraints. As Karanja is a cross pollinated plant, pollinators activity, pollen viability, stigma receptivity and nectar availability are major impact on pod set and oil production. Further, there are many insects which visit the flowers but Megachilid bees are the one which visit more frequently and they could be the major pollinators of Karanja. Floral biology is the base to study the pollinators and their role in pod set and this knowledge will help us to overcome yield constraints and increase the biofuel production.

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Conflict of Interest
Author declares that there is no conflict of interest

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