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Floral development and potential pollinators of *Syzygium myhendrae* (Bedd. ex Brandis) Gamble, a wild endemic tree of the Southern Western Ghats, India

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Abstract

Syzygium myhendrae, is a semi-evergreen, endemic and endangered tree species of the Southern Western Ghats, India which exhibits bi-annual mode of flowering and fruiting behaviour. This research is specifically aimed to observe the sequence of morphological processes occurring during floral development and to identify the potential pollinators from the different floral visitors. These aims are achieved by examining the individual trees of candidate species during their flowering season. Sticky traps, bee bowls, sweep nets and pan traps were used to capture floral visitors at the anthesis stage. Visitation frequency and visitor activity index were calculated to distinguish effective pollinators from visitor insects. The results showed nine stages in flower development starting from flower bud emergence to fruit ripening, which took 6-9 weeks. Nine species of insects were recorded as visitors. Honey bees (*Apis cerana*, *A. dorsata* and *T. iridipennis*), butterflies (*Hypolimnas misippus* and *Pachilopta pandiana*), wasp species, beetle species, fly species were exclusively visiting the flowers of the candidate species. Among the honey bees, *A. dorsata* showed high visitation frequency (0.40±0.01) followed by *A. cerana* (0.31±0.02) and *T. iridipennis* (0.52±0.02). As per the visitation frequency, it can be concluded that *A. dorsata* was the most frequent and effective pollinator.

Keywords

Anthesis; endangered; floral visitor; honey bees; visitor activity index

Introduction

Syzygium myhendrae (Bedd. ex Brandis) Gamble is a lesser-known tree species of the Myrtaceae family and is considered as an ornamental plant (1). *Syzygium* is the largest genus of the Myrtaceae family comprising of 1200 species which have a wide distribution in tropical and subtropical regions (2). Generally, the *Syzygium* genus is used in various aspects. Several *Syzygium* species are widely known as fruit-producing plants. They are *S. samarangense* (wax apple/jambu semarang), *S. aqueum* (water apple), *S. polycephalum* (kopo/kupa), and *S. malaccense* (malay apple). *S. aromaticum*, *S. polyanthum,* and *S. cumini* are known as producers of medicinal raw materials and essential oils (3).

The flower is an essential part of a plant and plays a vital role in reproduction. As a result of the adaptation process of certain plant species, flowers have acquired different shapes, colour and have become helpful to

the pollinators for successful pollination (4). Floral development consists of five stages. They are flower induction, bud initiation, pre-anthesis, anthesis, pollination and fertilization. Nevertheless, depending on the interaction between internal (phytohormones and genetic characters) as well as external factors (temperature, light intensity, humidity, and minerals), these phases are different among species (5). Due to the attractive young leaves and flowers, *S. myhendrae* can be introduced as an ornamental plant (6) and it has the potential to provide ecosystem service, mainly by acting as a source of insect food which also helps to maintain a mutually beneficial relationship between pollinators and plants (7). The details on flower development, identification of pollinators and their behaviour is indicative of the breeding system and its efficiency which in turn explain the gene flow systems and find their application in conservative strategies. Information about the insect pollinators is useful for the sustainable use of this plant.

The present study was aimed at observing the phases of flower development and visiting insects of *S. myhendrae*. The study also aimed to determine the legitimate pollinators from the visiting insects.

Materials and Methods

Study area

The research was conducted in 2017-2020, during the flowering seasons of *S. myhendrae*. Ten individual trees of about 12-20 m. in height were randomly selected for the present investigation. The research location was at the shola forest areas of Ponmudi (800-1000 m. asl 10012'29.60" N & 7028'50.32" E), part of the Agasthyamala biosphere reserve. The climatic pattern of the study site was shown in Fig. 1. The study site has a Type C climate (based on Schmidt and Ferguson's). The study site prevails heavy southwest monsoon and minimum light intensity. The wind velocity of 45 km/hr was also recorded during the study (Fig. 1). Herbarium specimens were deposited in TBGT herbarium (Collection number:70627, Accession number:40843).

Field studies

Field observations were conducted to observe the various flowering stages. From the date of bud emergence, the developments were regularly observed and photographs were taken. During the blooming phase, flowers were freshly collected and morphological characters were recorded by using a stereomicroscope and a hand lens. A digital Vernier caliper was also used to measure the size of floral parts (8).

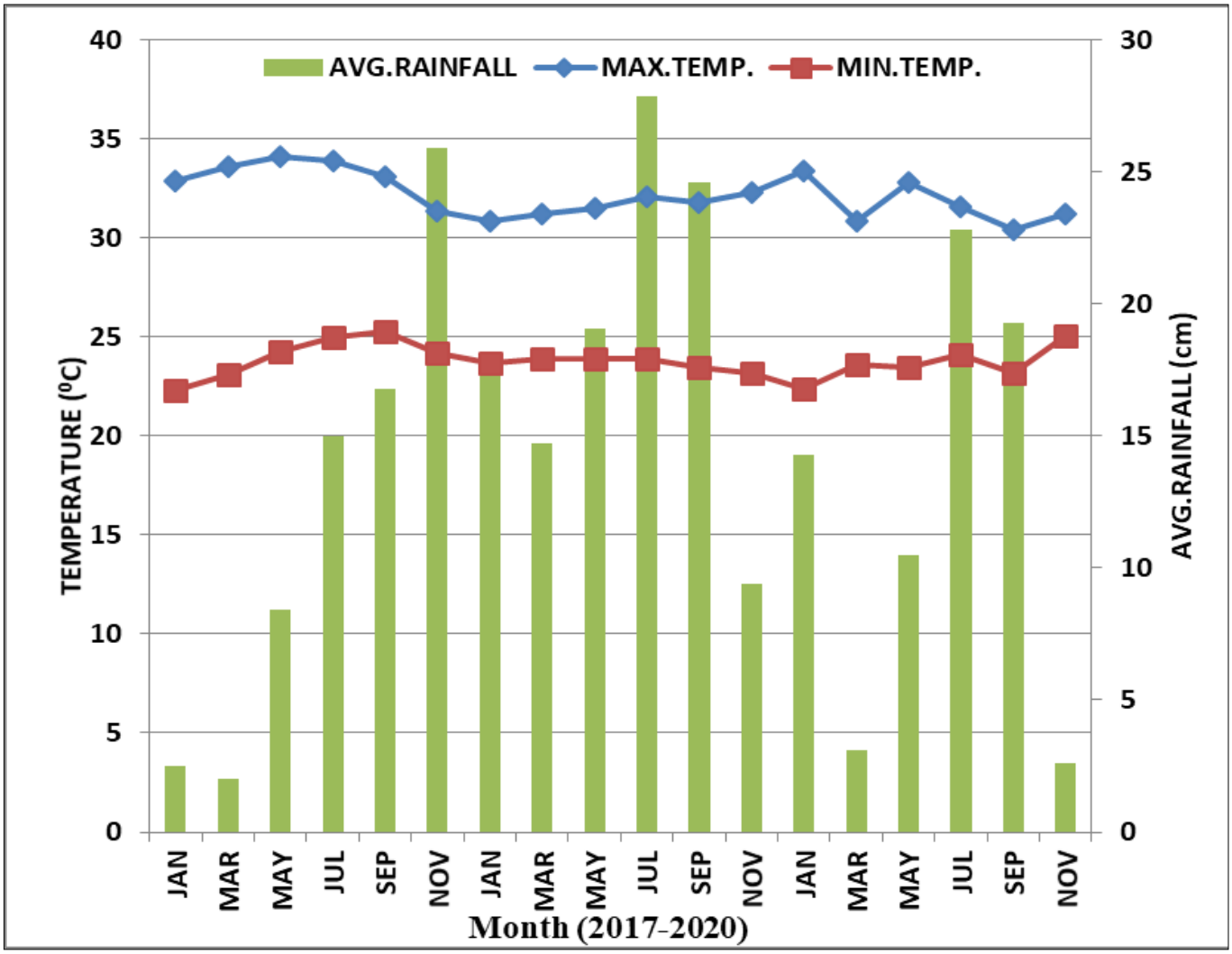

Fig. 1. Ombrothermic diagram for the study area means the monthly minimum and maximum temperature (**ºC**) and rainfall (**cm**) during the study period (2017-2020).

Observation of floral visitors and pollinators

Insect specimens were captured for identification by netting them as they foraged at flowers, and depositing them individually in labelled plastic bags. Sweep net sampling (Fig. 2 E), pan traps, bee bowls (Fig. 2 C & D), and sticky traps (Fig. 2 A & B) were also used. In sweep net sampling, sweeping was done on the areas with more flowers. Yellow -coloured shallow trays filled with 5% soap solution were used as pan traps. Bee bowls were prepared by using small ice cream cups which were painted on the inside with fluorescent yellow, blue, and white paints. 24 bowls (8 of each colour) were placed five meters apart and randomly numbered. Considering the height of the tree, about PVC pipes of 90 cm length and 6 cm diameter were fixed in the field. The bee bowls were placed over the pipes and kept for 24 hr on the day of sampling. Bright yellow colour (550-600 nm wavelength) boards with sticky surfaces were used as sticky traps which were hung up to 5-10 cm away from the inflorescence. The insect traps were also kept for 24 hrs. in the field. At the end of the day, insects were killed by placing a few drops of ethyl acetate on a piece of rag in the bag and then leaving them for 2 hr The specimens were subsequently brought into labs, pinned and identified under a dissecting microscope using the keys in the insect manual and also by expert consultations (9).

Pollinators and floral visitors were distinguished by calculating the visitor activity index (VA). VA= [(A×B×C) + (A×B×D×E)]/2 is the formula used to estimate the visitor activity index. A×B×C denotes pollen transference and A×B×D×E represents flower visitor adaptation, attractiveness, and constancy (8). According to Ramirez, A (criteria number 1): Copious amount of pollen from the visiting plant was considered as 1 for abundant, 0.5 for scarce, and 0 for no pollen present. B (criteria number 2): During the pollination process whether the body part of the visitor which carries pollen grains has an association with orientation or position of the sexual organs of the flowers which consists of 1 as satisfactory and 0 as non-satisfactory. C (criteria number 3): Whether the pollen load on the visitor's body has contact with the receptive stigma. D (criteria number 4): Flower size and floral visitor size relationship (1-satisfactory, 0-non-satisfactory). D (criteria number 5): Relative abundance of the visiting insect species. If the visitor activity index value comes between 0-1, the visitors were categorized as pollinators (7). Floral visitors, visitation frequency, visitor activity index were enlisted in Table 1.

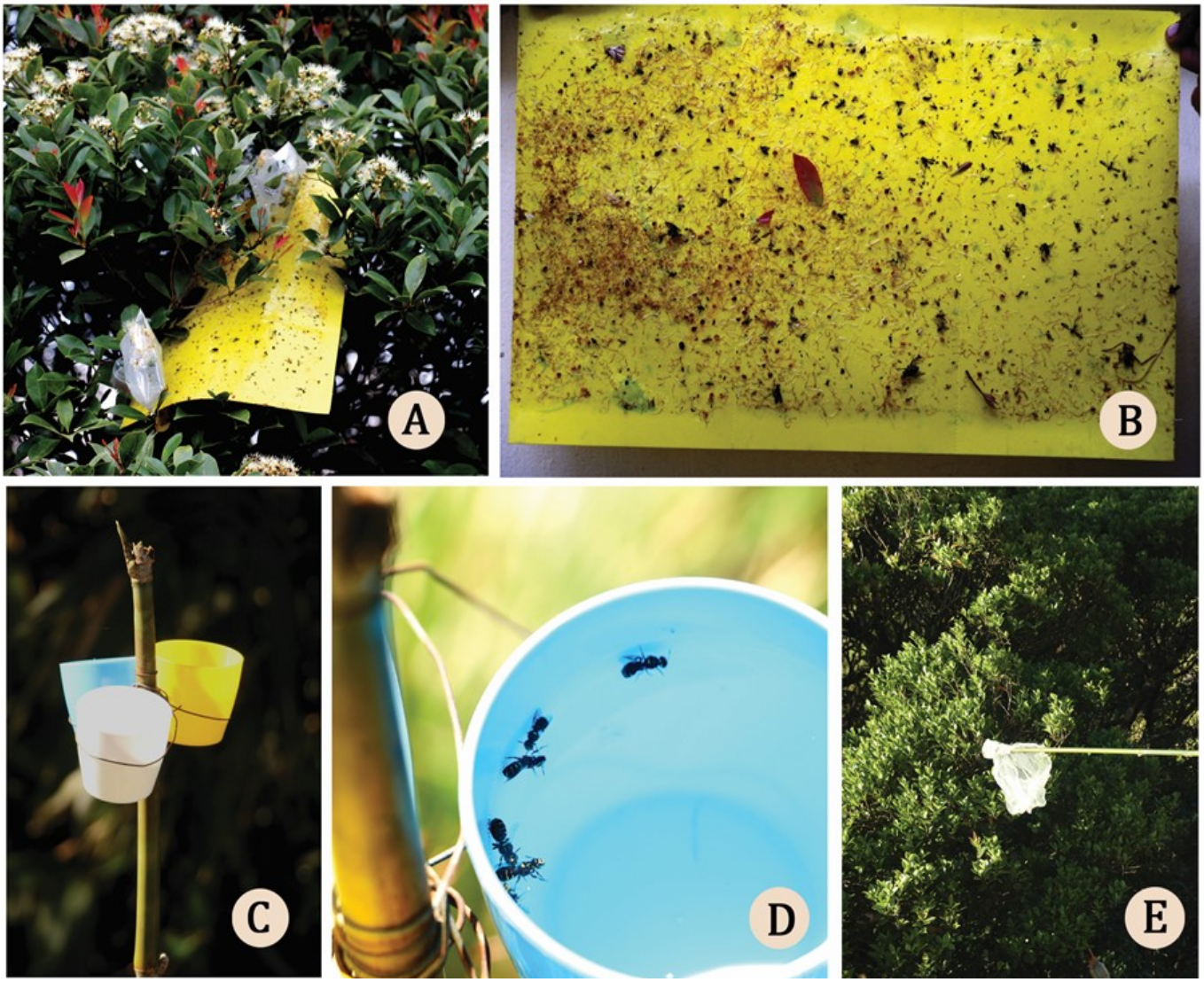

Fig. 2. Collection of pollinators . (**A**) & (**B**) Sticky traps, (**C**) &(**D**) Bee bowls, (**E**) Sweep net

Table 1. Floral visitors and pollinators in *Syzygium myhendrae*.

Sl.No	Visitor name	Nature of forage	Visitation frequency	Visitor activity index	Stigma touch	Pollinator/floral visitor
1	*Apis cerana*	P+N	0.31±0.02	0.65±0.01	+++	Pollinator
2	*Apis dorsata*	P+N	0.40±0.01	0.71±0.02	+++	Pollinator
3	*Tetragonula irridipennis*	P	0.18±0.01	052±0.02	++	Pollinator
4	*Pachilopta pandiana*	N	0.22±0.03	0.115±0.003	++	Floral visitor
5	*Hypolimnas misippus*	N	0.18±0.005	0.130±0.001	++	Floral visitor
6	Moth sps	N	0.25±0.01	0.237±0.002	+	Floral visitor
7	Fly sps	N	0.13±0.02	0.048±0.05	+	Floral visitor
8	Beetle sps	N	0.14±0.02	0.107±0.001	+	Floral visitor
9	Wasp sps	N	0.22±0.03	0.60±0.001	++	Pollinator

P-Pollen, N-nectar, +++-very good, ++-good, +-normal

Results

Morphological characters

Syzygium myhendrae is a medium-sized tree. Leaves are simple, oblanceolate, or obovate, exstipulate, apex obtusely acuminate. Young leaves are red and as it matures colour becomes green. Inflorescense is terminal corymbose cyme of umbellules. Peduncles are quadrangular. Sepals and petals are distinguishable and they are fused to form a calyptra over the bud. Morphological characters were enlisted in Table 2.

Table 2. Observations on morphological characters of *S. myhendrae*.

No.	Floral characters	Observations
1	Plant height	12-20 m
2	Inflorescence	Terminal corymbose cyme
3	No. of flowers/ Inflorescence	39±2.94
4	Flower size	8-9 mm
5	Tepal colour	White
6	Number of stamens	56-64
7	Length of stamens (mm)	4.81-5.42 mm
8	Length of the hypanthium	2.4-2.9 mm
9	Fruit shape	Globose
10	Size of the fruit	9.84±0.03×10.64±0.03 mm
11	Number of seeds	1
12	Pollen - ovule ratio	21,425:1
13	Length of style (mm)	3.10-3.52 mm

Flower development of Syzygium myhendrae

Flowering stages (Fig. 3 A-I) were observed and noted in Table 3.

Visitor insects and pollinators

Insects belonging to Hymenoptera, Diptera, and Coleoptera orders were identified as floral visitors as well as pollinators. During the peak period of flowering, two *Apis* species (*A. cerana* and *A. dorsata*), one stingless bee (*Tetragonula iridipennis*), two species of butterflies (*Pachilopta pandiana* and *Hypolimnas misippus*), one moth species, one fly species, one beetle species and one wasp species were recorded as floral visitors. All these insects are diurnal visitors. Visitation frequency was observed as high in honey bees. Among these honeybees *A. dorsata* (Fig.4 A, C & D) possess a high visitation frequency (0.40±0.01) followed by *A. cerana* (0.31±0.02) (Fig.4B) and *T. iridipennis* (052±0.02) (Fig.4 E&F). The visitation frequency of other floral visitors was recorded for the species *P. pandiana* (0.22±0.03) (Fig. 5 C & D), *H. misippus*(0.18±0.005) (Fig. 5 A & B), moth species (Fig. 6 E) (0.25±0.01), fly species (0.13±0.02) (Fig. 6 C), beetle species (0.14±0.02) (Fig. 6 D) and wasp species (0.22±0.03) (Fig. 6 A & B) respectively. When comparing with other visitors honey bees (*A. cerana*, *A. dorsata* &*T. iridipennis*) and the wasp species possess high visitation frequency and high visitor activity index. Their visitor activity index value comes between 0-1, and they carry enough pollen load and the body possesses a good orientation and contact with the flowers of *S. myhendrae*. So that honey bees and wasp species are considered as the pollinators of *S. myhendrae*. Other insects have low visitation frequency and low visitor activity index. However, the two butterfly species have good touch with stigma. But their body doesn't have enough pollen loads to carry out successful pollination. So all other observed insects were categorized as the floral visitors.

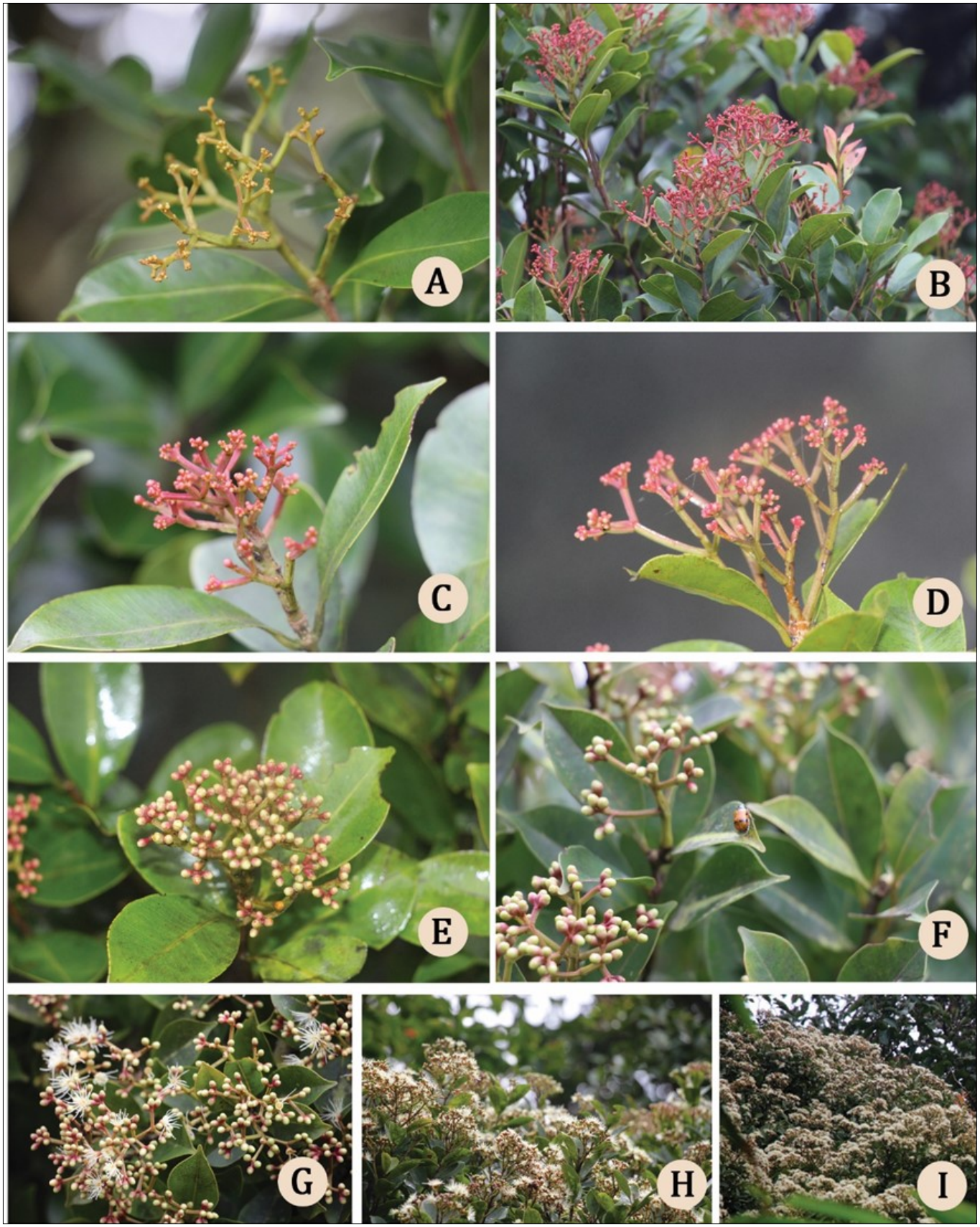

Fig. 3. Different stages of anthesis in *S. myhendrae*. (**A**) Flower bud emergence, (**B**) Peduncle extention, (**C**) & (**D**) Inflorescence elongation, (**E**) Flower bud enlargement, (**F**) Calyptra formation, (**G**) Starting of anthesis, (**H**) & (**I**) Anthesis

Finally, the results of the present research are expected to fill the information gap for *S. myhendrae* regarding the flower development, identification of potential pollinators and their behavior, so as to be able to develop this species for various potentials such as a service provider for the environment (a source of feed for pollinating animals) and as an ornamental plant etc. The details also help to develop better approaches in the characterization and identification of the candidate species.

Table 3. Flowering stages in *S.myhendrae*.

Sl. No	Flowering stages	Duration (days)
1	Flower bud emergence : Small flower bud starts to emerge	5
2	Peduncle extention: Peduncle starts to extend up to the formation of a second branch	7
3	Inflorescense elongation: Inflorescense get elongated up to four branches in the peduncle with the presence of more flower buds.	9-14
4	Flower bud enlargement: Peduncle growth ceases and flower buds get enlarged.	15-20
5	Calyptra formation: Sepals and petals fused together to form calyptras over the bud and buds were ready to bloom.	17-25
6	Starting of anthesis: Some flower buds starts to bloom, can be identified by the presence of tepals over the bud indicating the appearance of stamens and pistils	19-27
7	Anthesis: Stamens, pistils appear simultaneously with open tepals	22-29
8	Enlargement of the ovules: Cayptras fell off, flowers were in the basic shape, consists of pistil and ovule. Finally ovule become enlarged	23-33
9	Formation of fruits: Calyx along with gynoecium persists and gradually ovary develops in to fruit. Young fruits are green in colour	28-37
10	Ripening of fruits: Matured fruits turn into black purple colour with single seed	45-64
	Total time needed	6-9 weeks

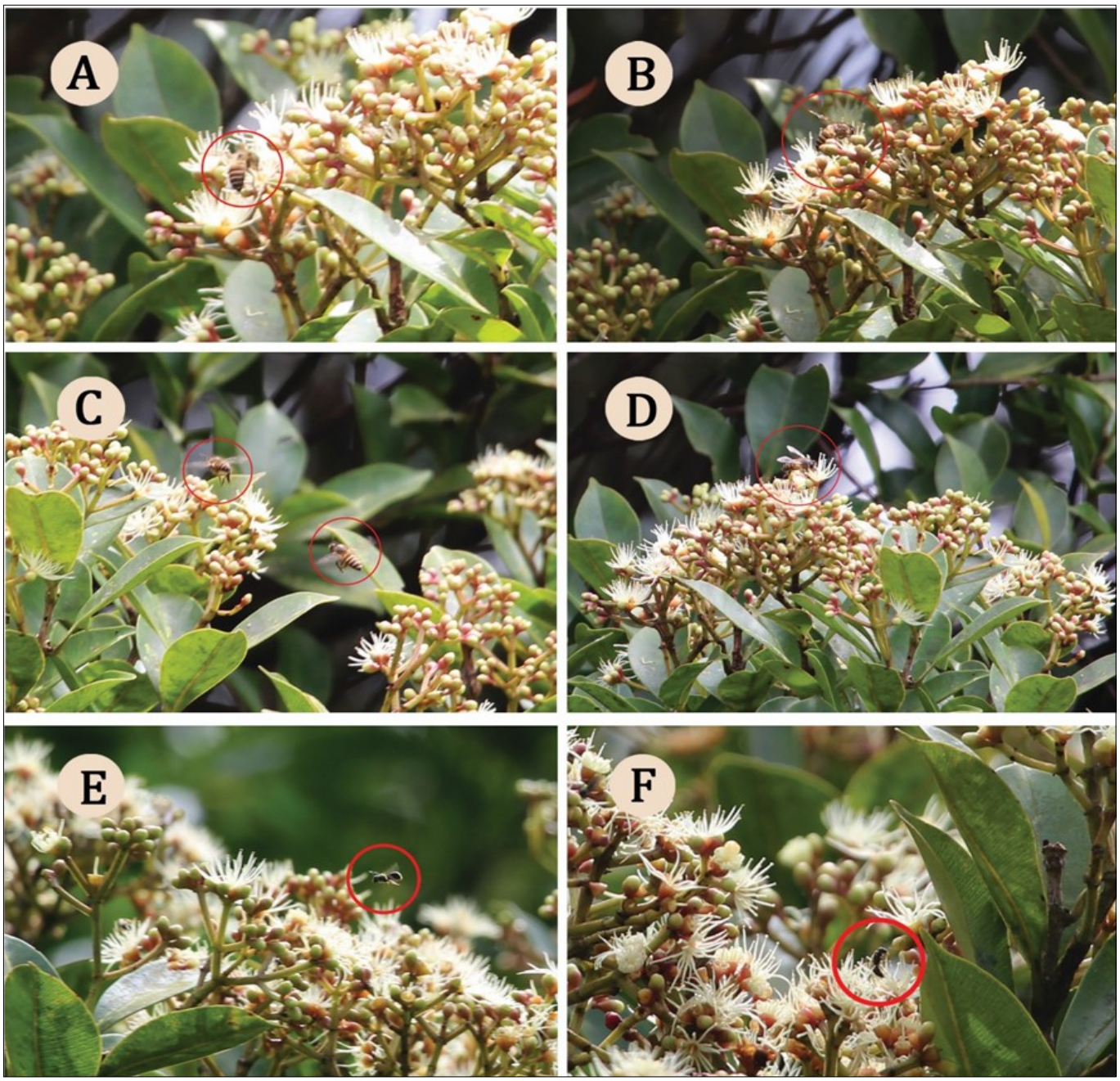

Fig. 4. Foraging activities of Honey Bees. (**A**), (**C**) & (**D**) *Apis dorsata,* (**B**) *Apis cerana*, (**E**) & (**F**) *Tetragonula iridipennis*

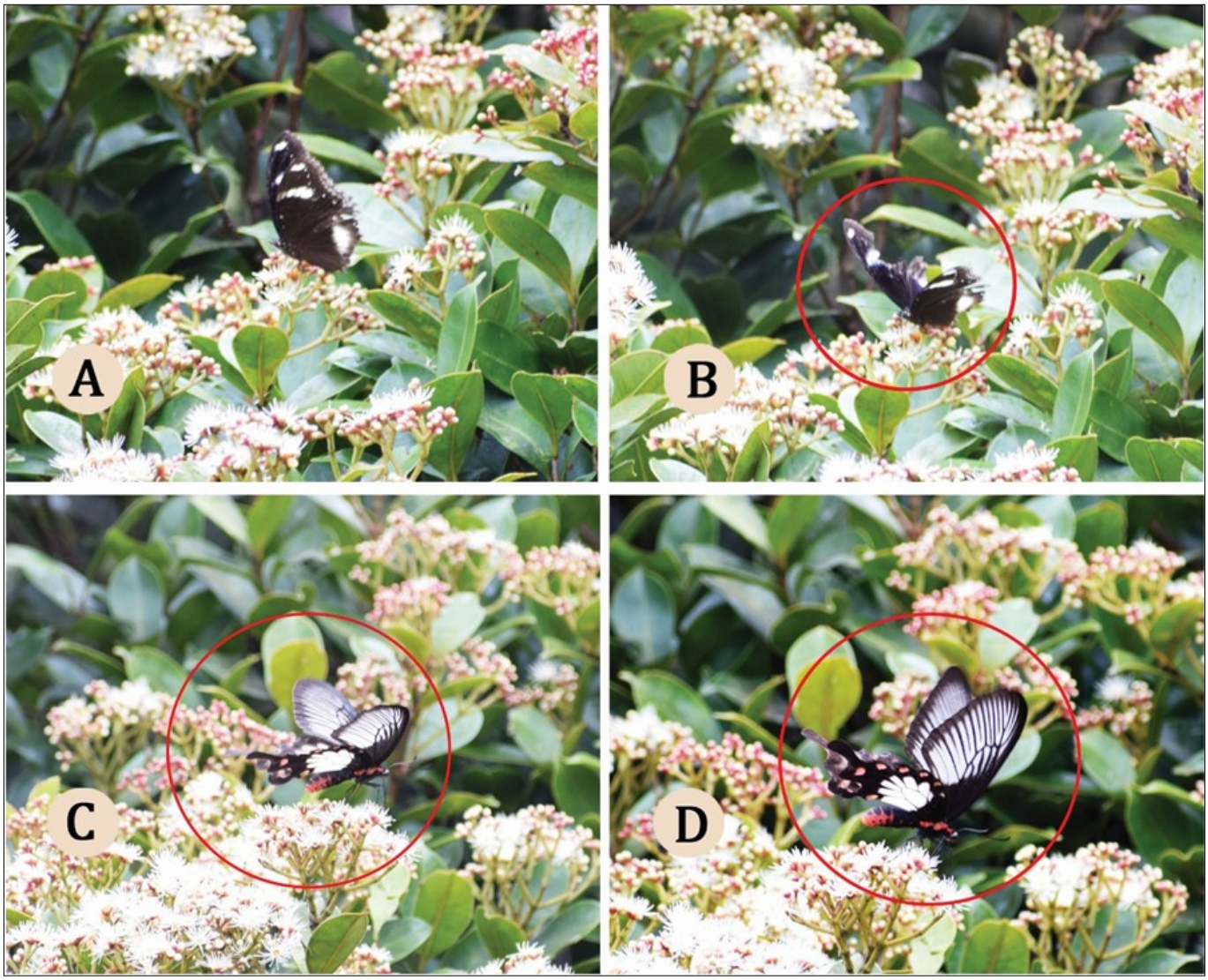

Fig. 5. Foraging activities of Butterflies in *S. myhendrae.* (**A**) & (**B**) *Hypolimnas misippus*, (**C**) & (**D**) *Pachilopta pandiana*

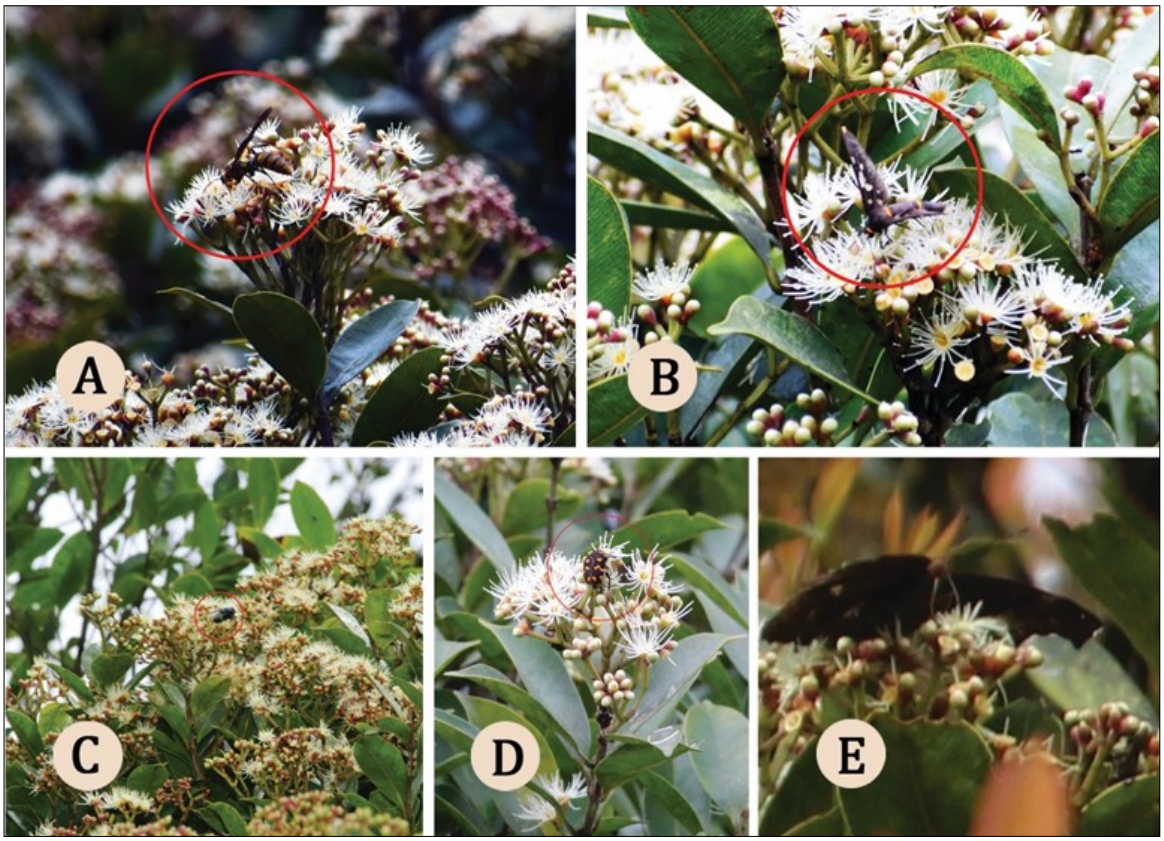

Fig. 6. Floral visitors of *S. myhendrae.* (**A**) & (**B**) Wasp sps., (**C**) Fly sps., (**D**) Beetle sps. (**E**) Moth sps.

Discussion

Syzygium myhendrae is a semi-evergreen plant that exhibits mass flowering. The flowering phase consists of nine stages and it starts from the emergence of potential tiny buds. It took almost 5 days to enter into the second phase of flowering, which is peduncle extension. Peduncles extend up to the formation of a second branch. After 7 days, inflorescence gets elongated with more flowers. It took 9-14 days for the inflorescence elongation. After 14 days of elongation, the flower bud becomes mature and anthesis starts with the presence of calyptras over the bud indicating the appearance of stamens and pistils which is the sixth phase of flowering. In the seventh phase, calyptras fell off and their stamens and pistils become exposed. After 2-3 days of life span calyx along with gynoecium persists and gradually develops into fruits. In the final stage, matured fruits turn into black purple colour. The flowering phase in *S. myhendrae* lasts for 6-9 weeks. *S. myrtifolium* flowering consists of ten stages and lasts for 105-124 days. According to the observation made by Erwin 2005, generally, there are six stages in flowering from the initiation to fruit development and seed formation. Similar studies have been done on several species of the *Syzygium* genus. *S. pycanthum* needs 80-89 days for the completion of flowering (10). *S. guineense* requires 116-128 days (2). *S. hirtum* needs 95-105 days (10). *S. caryophyllatum* needs 63-78 days (11) for the completion of entire stages of flowering. Even though these different species are in the same genus, they exhibit differences in flowering stages. Flowering processes are influenced by both internal as well as external factors (12). *S. caryophyllatum* flowering intensity varies from year to year. This is due to the inconsistent and low rain fall, which leads to change in the temperature and water availability. In majority of the woody species flowering and fruiting were observed in transition between wet and dry seasons (13). *S. myhendrae* trees flower at the onset of south-west monsoon.

S. myhendrae flowers possess calyptrate mechanism. Here sepals and petals are indistinguishable, they form calyptras over the bud. Flowers of the candidate species possess numerous stamens. These pollen grains act as a cue for the attraction of pollinators and also play an inevitable role in pollination. There are reports on the exhibition of plenty of stamens in *S.caryophyllatum* (11), *S.occidentale* (14), and *S.myrtifolium* (3). Legitimate pollinators of *S.myhendrae* were observed as hymenopteran members. A similar observation was made on *S. syzygioides* by Lack and Kevan. Boutler reported that in *S. sayeri* besides hymenopteran & dipteran members Thysanoptera and Araneida members also contributed to the pollination (4).

The role of pollinators is important for the candidate species, because its propagation takes place only through seeds. To produce seeds it is necessary to have pollination. Different studies have been conducted on several *Syzygium* species such as *S. alternifolium* (15), *S. occidentale* (16), *S. tierneyanum* (17), *S. caryophyllatum* (18) to identify different pollinators. In some *Syzygium* species camera trapping and video surveillance methods were used to identify the pollinators (19). Not only insects but also bats, birds, and possums were also reported as the pollinators of *Syzygium* species (20). According to Komamura, some visitors obtain pollen or nectar, however do not contribute in pollination (20). Detailed observations like visiting frequency, counting the number of pollen grains per body surface, calculating fruit set and investigating the mean number of seeds per fruit produced after a single visit of each visiting species are required to determine which flower visitor is a pollinator (20). In the present study, similar observations were made to determine the legitimate pollinators.

Conclusion

To conclude, the study has investigated the different flowering stages and floral visitors as well as pollinators of an endangered tree species *S. myhendrae*. The tree possesses eight stages from initiation to completion of flowering. Hymenopteran members are found to be the actual pollinators of the plant. Butterflies, wasps, beetles, and fly species were observed as floral visitors. As red listed plant species exhibit preferences ranging from bio-physical habitat requirements to pollinator specificity, every study on this plant is novel and necessary. This novel work on the reproductive dimension of the red-listed plant can reveal the factors leading to limited distribution of the plant and act as a frame work for formulating conservative measures through propagation.

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Authors contributions

DSP and AKS planned the experiments. DSP collected the plant materials and performed the experiments under the guidance of AKS. Both authors analyzed the research data and drafted the manuscript.

Compliance with ethical standards

Conflict of interest: The authors do not have any conflict of interest to declare.

Ethical issues: None.

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