PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.2392

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RESEARCH ARTICLE

# Floral biology and pollinator captivating semiochemicals in *Goniothalamus wynaadensis* (Bedd.) Bedd. (Annonaceae) an endemic tree species of the Western Ghats

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OPEN ACCESS

**ARTICLE HISTORY**
Received: 24 February 2023
Accepted: 23 May 2023
Available online
Version 1.0 : 28 June 2023

Check for updates

**Additional information**

**Peer review**: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Jayan A, Sreekala A K. Floral biology and pollinator captivating semiochemicals in *Goniothalamus wynaadensis* (Bedd.) Bedd. (Annonaceae) an endemic tree species of the Western Ghats. Plant Science Today (Early Access). https://doi.org/10.14719/pst.2392

## Abstract

*Goniothalamus wynaadensis* (Bedd.) Bedd. (Family Annonaceae) is an endemic tree species growing in the evergreen forests of the Western Ghats up to 900m asl. The present study aims at understanding the functional floral traits and semiochemicals found in *G. wynaadensis* which support plant-pollinator interactions. The study revealed *G. wynaadensis* exhibits abbreviated anthesis (c.28hrs). *Goniothalamus* flowers exhibit unique features of cantharophily. The study indicated that outcrossing is promoted by protogyny, and pollination efficiency is enhanced by pollen aggregation, anthesis duration, and pollinator trapping which involves a close alignment between petal movements and the circadian rhythm of pollinators. Curculionidae and Nitidulidae beetles are effective pollinators of the plant. In beetle-pollinated flowers, floral scent is a crucial component for pollinator attraction. *G. wynaadensis* has a strong fruity aroma. The floral scent analysis performed using headspace solid- phase microextraction combined with GC-MS detected various semiochemicals that support plant-pollinator interactions. Ethyl butyrate, isobutyl acetate, 3-hydroxyl-3-methyl-2-butanol, isopentyl acetate, and 3-methyl-1-butanol dominate the floral scent of *G. wynaadensis*.

## Keywords

Annonaceae; circadian trapping; floral scent; cantharophily; semiochemicals

## Introduction

*Goniothalamus wynaadensis* is a near-threatened species endemic to the southern Western Ghats. *G. wynaadensis* is found primarily in Coorg area Karnataka, as well as Wayanad, Kozhikode, Kannur, and Malappuram districts in Kerala (1). With approximately 130 species, *Goniothalamus* (Blume) Hook.f & Thomson is one of the largest palaeotropical genera in Annonaceae (2). Mostly pollinated by beetles, with characteristic smells, feeding tissues, and structural protection of reproductive organs typical of specialized cantharophilous flowers, Annonaceae is considered the most successful of primitive angiosperms. This specialized cantharophily emerged towards the end of the Jurassic or the start of the Cretaceous, possibly in conjunction with the diversification of typical flower beetles (3-5).

Flowers of the genus *Goniothalamus*, which are mostly pollinated by beetles, display distinct cantharophily features. The genus is distinguished by its hanging axillary (or slightly supra axillary) flowers. The flowers, like other Annonaceae, have three sepals and two whorls of three petals. The

outer petals of the *Goniothalamus* are frequently larger than the inner petals, and the inner petals conceal the reproductive organs, generating a mitriform dome. The flowers are bisexual, with numerous free stamens and carpels. The stamens contain broad, variable-shaped apical connectives that range from truncate to apiculate with septate thecae (6) and pollen grains are discharged in units of tetrads (7, 8). The goal of the study was to understand the floral biology and the identification of floral semiochemicals in *G. wynaadensis.* We investigated the following questions:

1. Effect of floral morphology in circadian trapping of pollinators.

2. Identification of sexual stages of flower.

3. Estimation of stigmatic receptivity.

4. Pollen fertility and viability rates.

5.Identification of semiochemicals and their role in pollinator attraction.

## Materials and Methods

### Study area

The present study was carried out on plants of *Goniothalamus wynaadensis* growing in the evergreen forests of Periya Reserve Forest in Wayanad district (N 75°48′ 25.0", E 11° 50′ 50.0"), Thamarassery Ghat pass (N 11° 29′54.0" E 75°1′20.0") located in Kozhikode district and Nedumpoil (N 11° 51′14.4" E 75°44′48.12") of Kannur district, Kerala from 31 December 2019 to 1 January 2022. Plants were found at an elevation ranging from 350 to 900m asl. Voucher specimens of *G. wynaadensis* were collected from Thamarassery Ghat pass (Herbarium TBGT Coll No.88603), Periya Reserve Forest (Herbarium TBGT Coll No. 85459), Nedumpoil (Herbarium Coll No. 96603). All the specimens were deposited in Jawaharlal Nehru Tropical Botanic Garden and Research Institute Herbarium.

### Phenology

We tagged 75 flower buds of *G. wynaadensis* from 15 trees of each of the three populations monitored them regularly until the opening of petals (before the initiation of the pistillate phase). After anthesis, the observations were taken at 1-hour intervals at the end of the staminate phase over 10days in three populations. We recorded the observations and measurements based on floral morphological changes and floral scent production.

### Floral morphology

We studied the floral morphological characters of selected species along with the reproductive mechanism. Also, visually assessed the colour changes in the essential and non-essential whorls of the flower during various developmental stages. Duration of each developmental stage, separation and closing of petals, and wilting and abscission of floral parts were studied. Floral measurements were taken by using a digital vernier calliper (Mitutoyo Absolute Digimatic - Japan made). For this purpose, we collected 50 mature flowers (at and around the pistillate stage) from the field and preserved them in 70% ethanol.

### Identification of the sexual stages of the flowers

To identify the sexual stages of the flower, we assessed the duration of receptivity, the timing of the receptive phase of stigmas and smell release, presence and absence of stigmatic exudates. Stamens were observed to check the dehiscence of the anther as an indication of the staminate phase of the flower. Timing of the anther dehiscence, duration of pollen availability, presence of reserve food materials such as starch and lipid in the pollen were also studied. Pollen viability along with color changes of stamens during all developmental stages was also studied.

### Estimation of stigmatic receptivity

#### $H_2O_2$

We estimated the stigmatic receptivity by immersing the stigmas in a 3% hydrogen peroxide ($H_2O_2$) solution and examined for bubble formation (9).Bubbles formed as a result of the activity of peroxidase enzymes, and are considered indicative of receptivity. The stigmatic receptivity was estimated by assigning various degrees of receptivity: (-) no reaction, (+) weak positive reaction, (++) strong positive reaction, (+++) very strong positive reaction (10).

#### *Localization of esterase on the stigma surface*

We used the methodology proposed by Shivanna (11) for this analysis. Ten matured flowers in pre-pistillate, pistillate, interim and staminate phases were selected. The floral parts, except the pistils were removed and the excised pistils of selected stages were dipped in solution A and solution B separately and incubated at 25°C in a humidity chamber for 30minutes. Solution A was prepared by adding 5mg of α-Naphthyl acetate (substrate), 10ml of phosphate buffer, 10% sucrose and 25mg fast blue. α- Napthyl acetate is insoluble in phosphate buffer, hence it is first dissolved in a few drops of acetone in a screw-cap bottle and then added the buffer, sucrose, fast blue B and are mixed thoroughly. Solution B (control without substrate) was prepared by combining 10ml phosphate buffer, 10% sucrose and 25mg fast blue. After the specific incubation period, the stigmas were removed and washed with phosphate buffer (pH 6.8). The stigmas were mounted in 50% glycerin and observed under the light microscope (Nikon Eclipse 5Oi) and details of the stigmatic surfaces incubated in both solutions A and B were compared.

### Pollen fertility

We assessed pollen fertility by the acetocarmine glycerine staining technique (11). For this purpose, fresh pollen grains were collected from the different flowers and transferred to a clean slide. Added two drops of acetocarmine glycerine mixture (3:1) and mixed thoroughly. After 15minutes the slides were examined under Nikon Eclipse 50i light microscope. The numbers of stained and unstained pollen grains were counted. The stained pollen grains were considered fertile whereas the unstained pollen grains were counted as sterile.

### Pollen viability by the Fluoro-chromatic reaction (FCR) test

We prepared the stock solution of the Fluorescein Di-Acetate (FDA**)** by adding 10 mg of FDA to 5 ml of acetone. The test solution was prepared by adding a few drops of stock solution

to 1 ml of 10% sucrose solution until the turbidity persisted. Pollen grains were mounted in a drop of FDA solution and incubated for 5 minutes and the preparation was observed under a Nikon Eclipse 50i fluorescence microscope. Bright green fluorescing pollen grains were counted as viable (12).

### Pollen viability by DAB test

We dissolved sigma fast 3.3 di-amino benzidine (DAB) tablets in 1ml distilled water in a clean vial. The solution was shaken thoroughly, and the pollen grains collected from different anthers were suspended in 5-10µl of the solution. The pollen grains were mixed well and allowed to dry. The sample was incubated for 5min and warmed slightly. The slides were sealed with cover glass and observed under the microscope. The stained pollen grains (brown coloured) were counted as viable which indicated the presence of peroxidase in pollen grains (17).

### Pollen histochemistry

The pollen samples were immersed in a drop of IKI (Iodine in potassium Iodide) solution or a drop of Sudan IV and examined under a microscope. A brown color indicated the presence of starch and red color indicated the presence of lipids in the pollen grains (3).

### Assessment of floral visitors

We carried out the observations of floral visitors from the beginning of the pistillate phase to the end of the staminate phase in a total of 75 flowers (25 flowers each belonging to three different populations). The duration and frequency of insect visits in each flower were recorded. Potential pollinators were identified after checking the presence of pollen grains attached to the body of the floral visitors. All the insects collected were transferred into small vials containing 5ml of ethanol. The vials are then shaken vigorously for 2 minutes to detach the pollen grains from body of insects. The insects were taken out and the residue was allowed to evaporate. Pollen grains were observed under a microscope. We used the following criteria to identify effective pollinators i) their arrival coinciding with functionally active stages of flowers, ii) higher visitation rates, iii) their movements to other flowers of the same species. The insects found inside the floral chamber were fixed in 70% ethyl alcohol and photographs were taken. Pollinators were identified by Dr Chris Lyal, Research entomologist, Natural History Museum London and Gareth Powell, Department of Biology, Brigham young university.

### Floral scent chemistry

Scent–producing flowers were collected using previously unused polypropylene bags; the bags were sealed to limit air movement.

#### *GC MS Procedure*

GC-MS analysis of floral volatiles was performed on a Shimadzu QP 2020 NX series gas chromatograph (Kyoto, Japan) fitted with an SH-Rxi-5Sil MS (1,4-bis(dimethyl siloxane) phenylene dimethylpolysiloxane, 30m×0.25mm, i.d., 0.25µm film thickness, Restek, USA) capillary column-MS operation conditions: Inject mode: split (split ratio: 50:1); injector temperature, 240°C; Interface, 260°C; oven temperature-programmed, 60-250°C (3°C/min); carrier gas, Heat 1.4mL/min. Mass spectra: Electron Impact (EI +) mode, 70eV with a mass range of 50 to 550m/z; ion source temperature, 240°C.

## Result and Discussion

### Phenology

*Goniothalamus wynaadensis* is a small tree, 2.2-5m tall. This species grows in the understory tree layer of the evergreen forests of the southern Western Ghats. Floral bud initiation starts at the end of September, along with leaf bud initiation. From bud initiation to anthesis (flower development period), it takes almost 93–103days. Flowering season starts in December and extends up to half of February. Peak flowering season is from mid of December to mid-January. Fruit initiation was observed in last April and maturation was found from November to December. Fruit is an aggregate of monocarps. Ripened fruits are orange to red in colour and the majority of fruits were found to be predated by Diptera larvae.

### Floral morphology

Flowers of *G. wynaadensis* are pendent, solitary and hermaphroditic. Three valvate sepals have an average length of 7.6±1.4mm. Six valvate petals are arranged in two whorls (Fig. 1A). All petals had a bright pinkish-to-red spot at their inner bases. The outer petals (20±3.17mm long, 11.2±1.7mm wide) were longer and wider than the inner petals (13.1±2.3 mm long, 8.47±1.47 mm wide). The inner three petals are apically fused with three apertures located at the base of the petal near the floral receptacle (Fig. 1B). These apertures are blocked by alternatively placed outer petals and thus forming a closed pollination chamber. The flower shows basal floral characteristics such as numerous stamens and pistils spirally arranged around the receptacle (Fig.1C), apocarpous gynoecium with superior ovary (Fig.1D), and hooded stamens (Fig.2E). Each stamen is 8.97±0.12mm long. The average number of stamens per flower is 90.14±6.52. The gynoecium is made up of multiple apocarpous and superior ovaries. Each carpel measures 6.07±0.21mm in length. A single ovule exists in each ovary. *G. wynaadensis* possesses a bifid stigma and, unlike other members of the Annonaceae family, an extended pseudo style is also seen.

### Identification of the sexual stages of the flowers

The flowers were protogynous, having a long pistillate stage and a brief pollen dispersal stage. The pre-receptive phase, receptive phase (or pistillate phase), intermediate phase, and staminate phase are the four major stages of flower development. The protogyny promotes outcrossing in Annonaceae (13). Phenological changes (Table 1) observed in each phase is as follows:

#### *Pre-receptive phase*

The outer petals change from green to ivory, and are compressed against the inner petal dome, blocking the chamber apertures and hence preventing pollinators from accessing the floral reproductive organs (Fig. 2A-D). The outer petals begin to rise, exposing the aperture 93 to 103 days after initiating the floral bud. Subsequent rising of petals occurs at c.06.00hr in the morning in *G. wynaadensis*.The outer petal rises from 03.00hr in *Goniothalamus tapisoides* Mat-Salleh and 06.00hr in *Goniothalamus suaveolens* Becc (13).

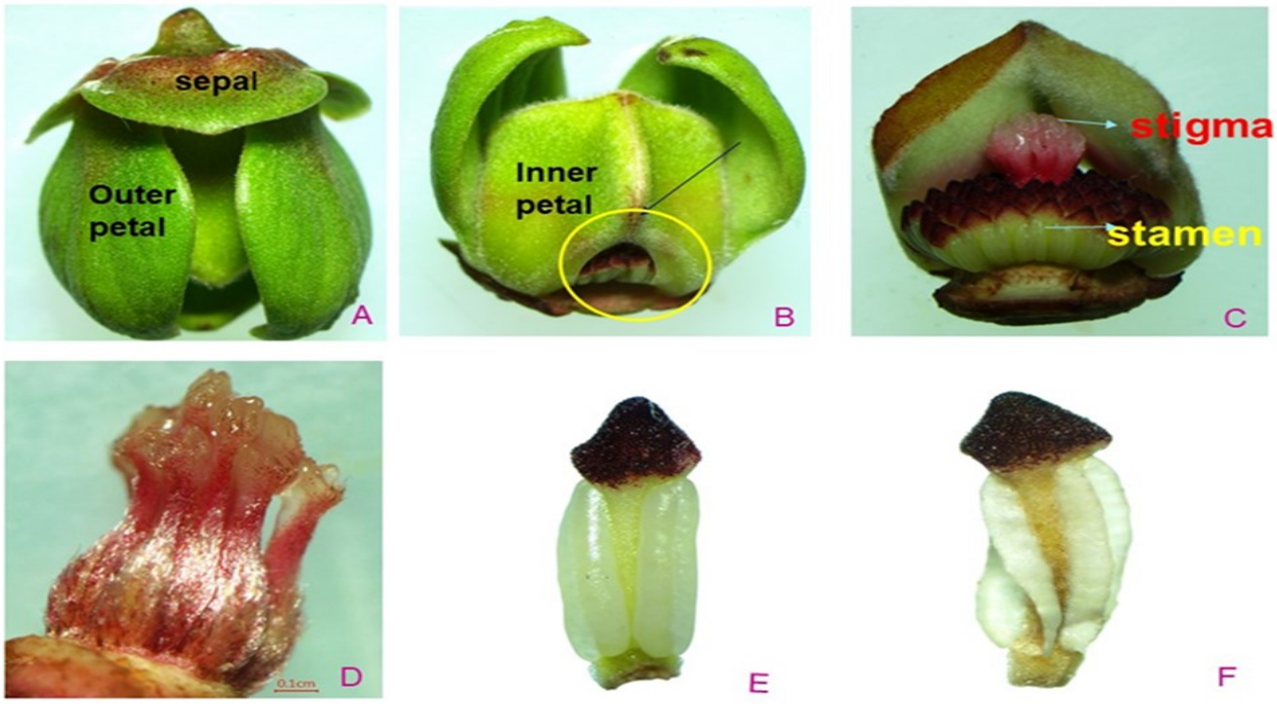


**Fig. 1.** Floral Morphology **(A)** Unopened flower buds with closed floral chamber, **(B)** Removal of outer petal exposing the opening of pollination chamber, (**C)** Arrangement of stamens and apocarpous pistil on torus, **(D)** Apocarpous gynoecium, **(E)** Stamen, (**F)** Mature stamen with longitudinal slits.

**Table 1.** Ethnomedicinal Plants Used for the Treatment of Diabetes by the Bhuyan Tribe in Sundargarh district, Odisha

| Phase | Floral stage | Duration | Outer petalposition | Sepal colour | Inner and outer petal colour | Stigma colour | Stigmatic exudate | Staminalhood colour | FruityScent |
|---|---|---|---|---|---|---|---|---|---|
| Pre-Pistillate stage | i | 90-100 days | Closed | Green | Green | Pink | Absent | Cream | Absent |
| | ii | 2 days | Gradually separate and clawed | Green | Green | Pink | Absent | Cream | Absent |
| | iii | 1 day | Gradually separate and flattened | Reddish | Ivory | Carmine | Present | Carmine | Absent |
| Pistillate stage | iv | 10 hrs | Fully separated resulting in the opening of the inner petal dome | Reddish | Ivory | Carmine | Present | Scarlet | Present |
| Interim phase | v | 8 hrs | Fully separated | Reddish | Ivory | Black | Dry | Scarlet | Present |
| Staminate phase | vi | 7 hrs | Abscission of corolla | Brown | Brown | Black | Dry | Scarlet | Absent |

### *Pistillate phase (c.10 hr duration)*

Flowers begin to open and become functionally receptive. As the outer petals expand out, the basal apertures of the receptive flowers become visible (Fig.2E). The release of a distinct fruity, pineapple-like aroma accompanies the pistillate phase. The stigmas were wet, and receptivity was indicated by releasing a small amount of sticky, sparkling exudate that linked the stigmas together as a single unit. During the receptive phase, the stigmas were carmine, while the stamens were light-yellowish cream with a scarlet staminal hood. The pistillate phase normally starts early in the morning (c.08.00hr) and concludes late in the evening of the first day (c.18.00hr). The outer petals return to their initial position on the evening of first day (c.17.00hr) by firmly appressing against the inner petal dome, and thus closing the opening of the flower. Thereby forming a closed pollination chamber which traps pollinators inside the flower (Fig. 2F). The fruity scent starts to diminish by evening of first day (c.17.00hr) before pistillate phase ends. The duration of pistillate phase is 13hr in *G. tapisoides* and 9hr in *G. suaveolens* (13), whereas 10hr in *G. wynaadensis*.

### *Interim phase (c.9hr duration)*

The next phase indicated by the drying of stigmatic exudate is known as the interim phase where the stigma is non-receptive, and the anther is still not dehisced. At this stage, the flower is considered sexually inactive. This phase lasted 9hours, clearly revealing that the flowers were protogynous. The colour of the petals and sepals of this phase were comparable to those of the pistillate phase, petals were ivory coloured and sepals were red in colour. The duration of interim phase is similar to previously reported in *G. suaveolens,* whereas duration of interim phase in *G. tapsioides* is 3hr.


**Fig. 2.** Different sexual stages in *Goniothalamus wynaadensis* **(A-D)** Pre pistillate stage, **(E)** Pistillate stage, **(F)** Interim phase represented by closed petals which aid circadian trapping of pollinators, **(G, H)** End of staminate phase represented by abscised corolla and stamens.

### *Staminate phase (c.7hr duration)*

Pollen grains were released from the anther through a longitudinal slit. At early morning of the second day (c.03.00hr), anthers dehisce. The petals and dehisced stamens abscise at c.10.00hr, while the sepals and ovaries remain attached to the torus. The pollinators arrived during the pistillate phase confined inside the chamber and were released concurrently with the abscission of the corolla (Fig.2G). Duration of staminate phase (7hr) is comparable to the staminate phase previously reported in *G. tapisoides* and *G. suaveolens* (13).

### Estimation of stigmatic receptivity

In dichogamous species, the length of stigmatic receptivity plays a critical role in regulating the isolation of male and female reproductive phases because stigma regulates the adhesion, hydration, and germination of pollen(14). Stigma receptivity is related to the activity of enzymes such as peroxidase, esterase and dehydrogenase(10). The stigmas immersed in $H_2O_2$ solution react with peroxidase on the stigma surface resulting in the release of air bubbles as an indication of receptivity. In our study, stigmas harvested one or two days before the pistillate stage (during the pre-pistillate stage) showed a highly positive response (++). Similarly, during the pistillate phase (Fig. 3A), a very significant positive reaction was found (+++), followed by a drop in receptivity during the intermediate and staminate stages. The reaction of α – naphthyl acetate allied with fast blue B salt revealed a dark red colouration (+++), mainly in the region of stigmatic lobes and pseudostyle, indicating the stigmatic receptivity (Fig.3B). When compared to other phases, the pistillate phase showed a relatively strong reactivity (Table 2). These findings were corroborated by the presence of stigmatic exudate, indicating that the flower is protogynous.

### Pollen fertility and Pollen viability

Pollen grains of *Goniothalamus wynaadensis* is monosulcate and dehisce as tetrads. Different types of tetrads were observed frequently from the genus *Goniothalamus*. Tetrahedral tetrad was reported in *Goniothalamus laticus*, tetragonal in *G. sawtehii*, *G. tamirensis*, *G. undulatus* and decussate in *G. repevensis* (8). Two tetrad types (Tetragonal and Decussate) were observed in *G. wynaadensis* in this study. Decussate tetrad has a mean LA (longest polar axes) of 139.167±20.534µm & mean SA (shortest polar axes) of 137.917±24.920µm (Fig.4A). Tetragonal tetrad has mean LA of 158.75±21.024µm and mean SA of 150.25±15.341µm (Fig. 4B).The acetocarmine- glycerin staining method revealed that 59.65±1.81 per cent of pollen grains were fertile (Fig. 5C).Pollen viability test by Fluorochromatic reaction (FCR) confirmed that pollen from freshly dehisced anthers showed the viability of 58.09±2.83 per cent (Fig. 5B). Pollen viability was also assessed by the Diaminobenzidine (DAB) test confirmed that 53.57±3.03 % of pollen grains were viable in the staminate phase (Fig. 5A).

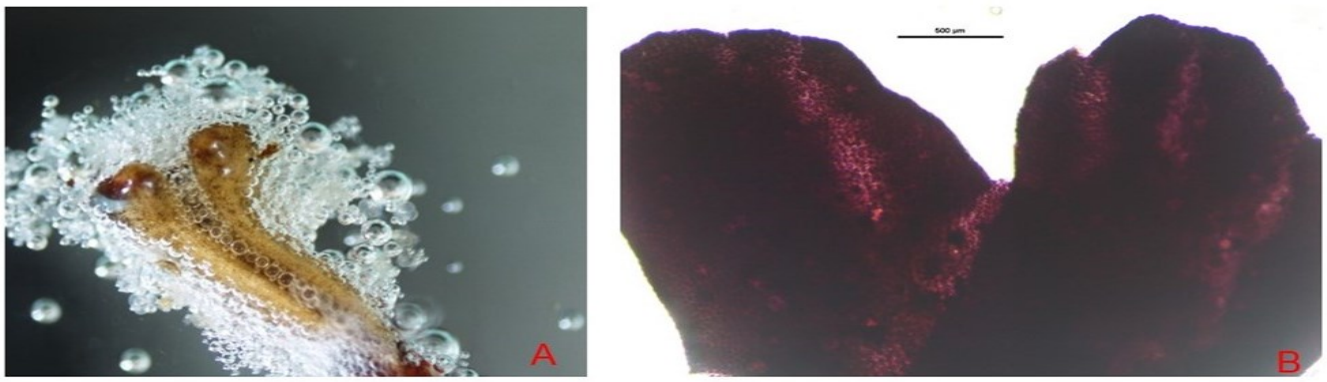


**Fig. 3.** *Goniothalamus wynaadensis* receptive stigmas at pistillate stage **(A)** Hydrogen peroxide test **(B)** Localized esterases on stigma surface.

**Table 2.** Results of stigma receptivity tests at different floral stages of the flower

| Floral Stages | Peroxidase activity | Esterase activity |
|---|---|---|
| Pre-pistillate | ++ | ++ |
| Pistillate | +++ | +++ |
| Interim | - | - |
| Staminate | - | - |

(-) no reaction; (+) weak positive reaction; (++) strong reaction; (+++) very strong positive reaction.

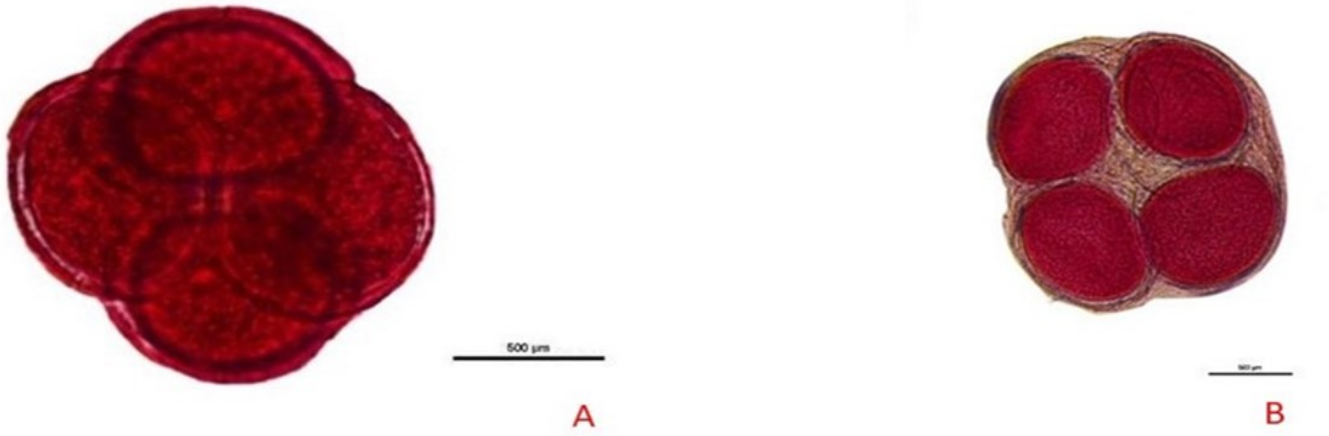


**Fig. 4.** Tetrad pollen grains of *Goniothalamus wynaadensis*; Decussate tetrad **(A)** and Tetragonal tetrad **(B)**.

## Assessment of floral visitors

The large majority of cantharophilous species of annonaceae are pollinated by small beetles (Nitidulidae and Curculionidae), with a body length up to 7mm (5). The floral arrangement in the genus *Goniothalamus* aids the circadian trapping of pollinators. Flowers of Goniothalamus exhibits a two-day flowering rhythm. When compared to *Goniothalamus suaveolens* Becc.'25-hour anthesis and *G. tapisoides* Mat-Salleh' 23-hour anthesis, *G. wynaadensis* exhibits 28-hour anthesis (13).It is hypothesised that pollinator trapping promotes effective inter-floral pollinator movement from staminate-phase flowers directly to pistillate-phase flowers because the timely release of pollinators coincides with the onset of the pistillate stage of other flowers (15, 16). The insect's foraging period coincides with the sexually active phases of the flower. The floral chamber of *G. wynaadensis* is open in the early morning (from 06.00hr) in *G. wynaadensis*.The floral scent production begins at c.08.00hr in the morning and intensifies at c.10.00 hr which acts as olfactory cue for the pollinator species. The beetles trapped inside the floral chamber is released on the second day of anthesis (c.10.00hr) enter directly to another flower at pistillate phase. In the present study, five floral visitors were found inside the floral chamber of *G.wynaadensis* viz, *Carpophilus* sp., *Urophorus* sp., *Colopterus* sp., Curculionidae sp., and green peach aphid. At c.10.00hr on the first day of anthesis, a group of *Carpophilus* beetles were discovered entering the floral chamber of *G. wynaadensis*, and the beetles, along with the abscission of the corolla, were discharged the next day at c.10.00hr (Fig. 7G, H). A single flower might contain up to 30 beetles at a time. This pollinator guild, together with pollen attached to their body parts, enters another flower of the same or different plant in the pistillate phase after spending 24 hours inside a solitary flower. During the staminate phase, *Carpophilus* beetles were found to feed pollen from *G. wynaadensis*. Many Annonaceae plants have pollen-feeding beetles (17). Beetles eat starch- and lipid-rich tissues (17). Our pollen histochemistry data showed that *G. wynaadensis* pollen grains are high in starch (Fig.6A) and

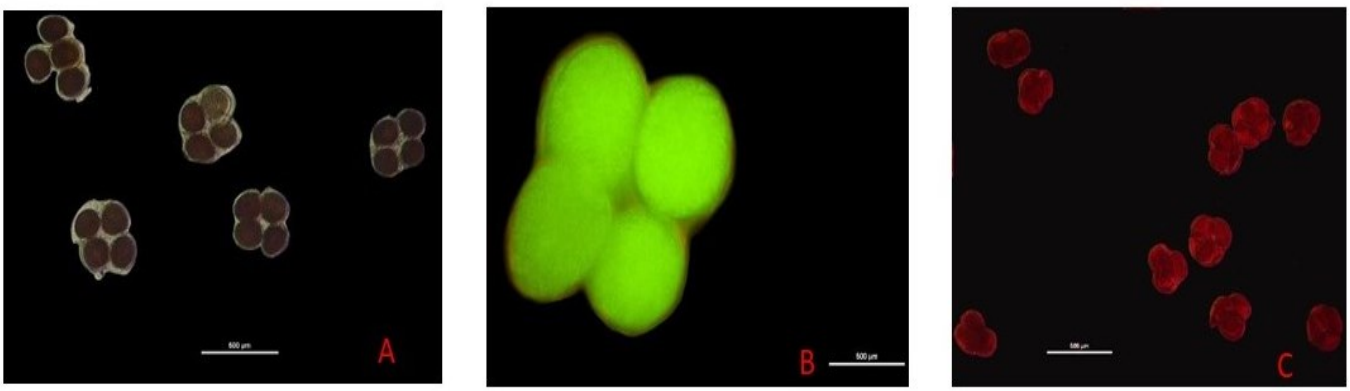


**Fig. 5.** Pollen viability and fertility tests; **(A)** viable pollen grains-stained brown by Diaminobenzidine test (DAB), **(B)** viable pollen grains stained green by Fluro chromatic reaction (FCR), **(C)** Fertile pollen grains stained red by Acetocarmine-glycerin technique.

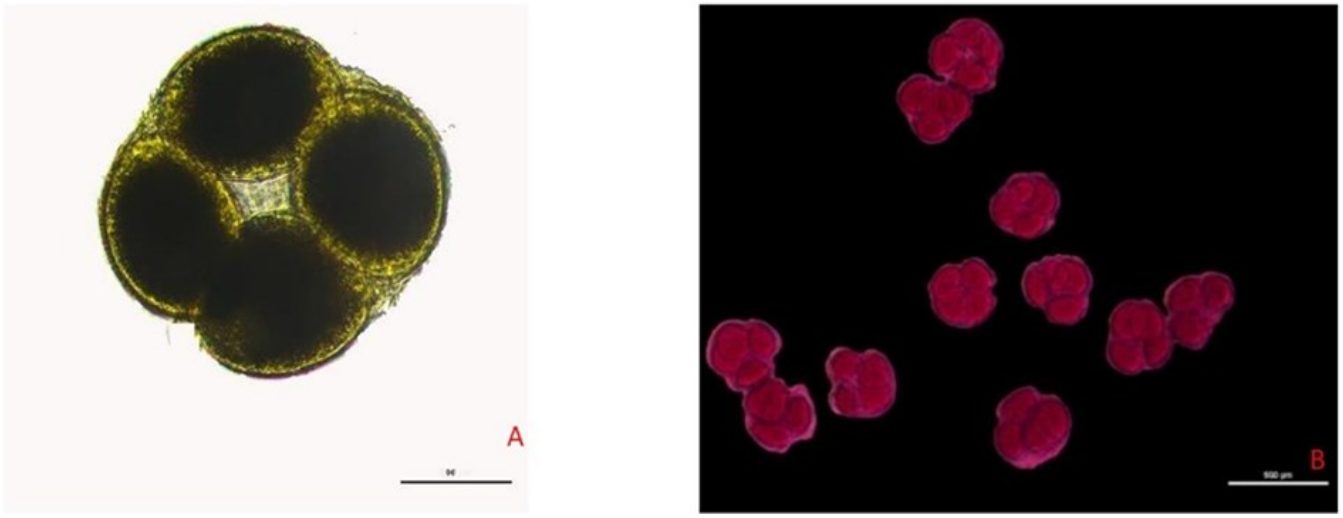


**Fig. 6.** Pollen histochemistry; **(A)** Starch localized in pollen grains by IKI test indicated by brown color **(B)** Lipids localized in pollen grains by Sudan IV test indicated by pink color.

lipid (Fig. 6B). The *Carpophilus* beetles were also reported as the major pollinators of *G. suaveolens* (13). During the reproductively active phase of the *G. wynaadensis* flower, another beetle species of Curculionidae, or little weevils, (Fig. 7C,F) were seen inside the floral chamber. A single flower has two or three weevils inside it. Brown-coloured holes were seen in the petals of the flowers visited by these insects, which were most likely caused by gnawing. The effective pollinators of *G. tapisoides* are reported as *Endaeus* beetles of Curculionidae (13). Other annonaceae members like *Polyalthia suberosa* (Roxb.) Thwaites, *Dasymaschalon trichoporum* Merr. were also reportedly pollinated by Curculionidae beetles (5). *Urophorus* (Fig. 7A, D) beetles, also known as pineapple beetles, are attracted to the *Goniothalamus wynaadenis* flower by its fruity scent. Inside a single flower around 25 beetles were found. They visit the flower during the pistillate phase and, get trapped inside the floral chamber and released during staminate phase. Another pollinator found inside the floral chamber of *G. wynaadensis* is *Colopterus* species (Fig. 7B, E) of Nitidulidae. Two to three *Colopterus* were found in a single flower during the reproductively active phase of *G. wynaadensis*. Three *Anaxagorea* species in the family annonaceae with a two-day flowering rhythm is also reported to be pollinated by *Colopterus* species (18). The green peach aphid was active on flowers of *G. wynaadensis* from c.08.00hrs to c.11.00hrs. The green peach aphid leaves the flower before chamber apertures are blocked by the movement of outer petals and the microscopic observations revealed the absence of pollen grains attached to their body surfaces. Hence, green peach aphid is only a visitor not an effective pollinator of *G. wynaadensis.* The *Urophorus* sp., *Carpophilus* sp., *Colopterus* sp., and Curculionidae sp., can be considered as an effective pollinator of *G. wynaadensis* as their arrival coincide with sexually active phases of flower and microscopic observations of these four beetles also revealed the presence of pollen grains of *G. wynaadensis* attached to their body. Among three populations studied, *Carpophilus* sp. and Curculionidae species are common to three populations. *Urophorus* sp. is found only from Periya Reserve Forest of Wayanad and *Colopterus* from Thamarrassery Ghat of Kozhikode.

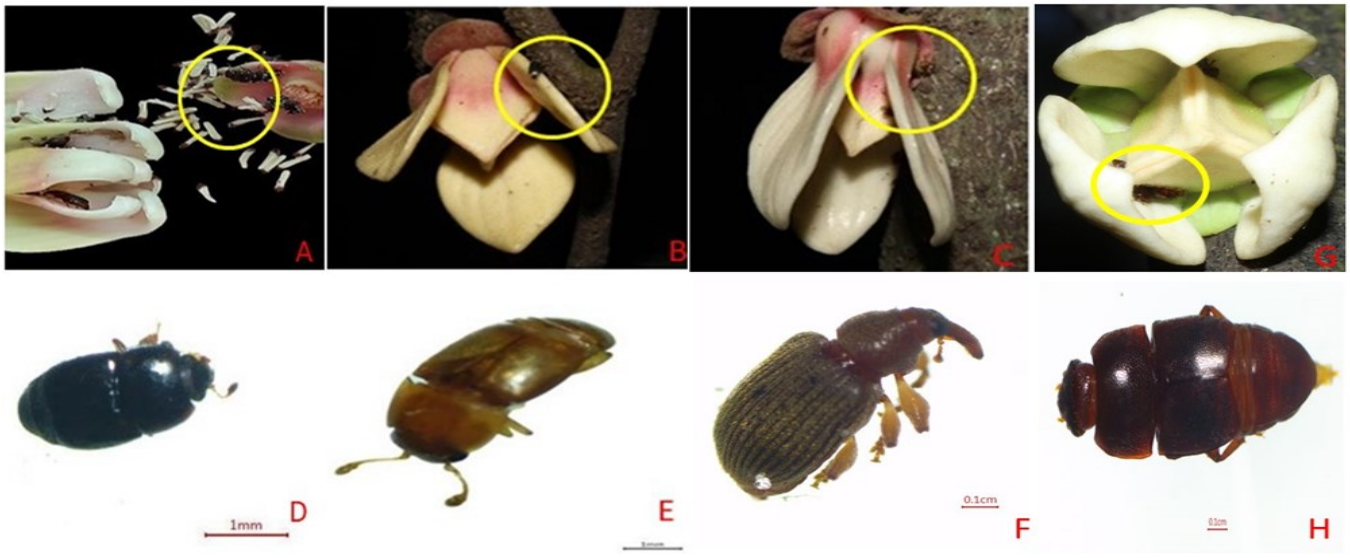


**Fig. 7.** Pollinators of *Goniothalamus wynaadensis*. **(A)** & **(D)** The genus *Urophorus*, **(B)** & **(E)** The genus *Colopterus*, **(C)** & **(F)** Curculionidae Species, **(G)** & **(H)** The genus *Carpophilus.*

## Floral scent chemistry

In beetle-pollinated flowers, floral perfumes are a crucial component for pollinator attraction(5).According to human olfaction, *G. wynaadensis* has a fruity aroma that smells like pineapple. Floral smell production begins at 08.00hrs during the pistillate phase and ceases at 20.00hrs during the interim phase. In this study we analysed the role of floral volatiles for the specific attraction of insect pollinators. Insects use various organic compounds to convey specific chemical messages that modify their behaviour and physiology. These organic compounds are known as semiochemicals. Volatile semiochemicals in flowers play a crucial role in attracting pollinators. Semiochemicals transmit information between individuals of the same species (pheromones) or different species (allelochemicals). Allelochemicals, depending on their functional role comprise kairomones (beneficial to the receiver), and allomones (beneficial to the emitter). The most well-known group of allelochemicals that act as kairomones are probably those involved in the location of food such as foraging kairomones, which are exploited by predators, parasites, parasitoids, herbivores and frugivores during their search for food and oviposition sites(19-21). The following semiochemicals were discovered using gas chromatography-mass spectrometry: methyl acetate,1-butanol, ethyl butyrate, isobutanol, isovaleraldehyde, 3-hydroxy-3-methylbutan-2-one, 3-methyl butan-1-ol,2-methylbutan-1-ol, ethyl isobutyrate, isobutyl acetate, ethyl 2-methyl butanoate, ethyl isovalerate, isopentyl acetate,2-methyl butyl acetate, isobutyl propionate, camphene, isobutyl isovalerate, butyl isobutyrate and caryophyllene (Table 3). Comparisons of floral volatile compounds of *G. wynaadensis* with those reported in previous research enable inferences regarding possible beetle attractants. Ethyl butyrate, isobutanol, 3-methyl-1 butanol, 2-methyl-1-butanol, ethyl isobutyrate,

**Table 3.** Semiochemicals for coleopteran pollinators of *Goniothalamus wynaadensis* identified through comparing the floral volatiles obtained by Gas Chromatography with Pherobase data base

| Compound Name | Retentiontime | RRI | Area% | Odour | Curculionidae | Nitidulidae | Other Coleoptera |
|---|---|---|---|---|---|---|---|
| Methyl acetate | 1.535 | 625.136 | 1.82 | Fruity, Blackcurrant | | | A |
| 1 Butanol | 1.619 | 632.758 | 0.93 | Fruity | A | | P, A |
| Ethyl butyrate | 1.823 | 651.27 | 50.73 | Fruity, Pineapple, strawberry, banana | K | A | A, K |
| Isobutanol | 1.881 | 656.53 | 2.33 | Alcohol, Licorice | | A, K | A, K |
| Isovaleraldehyde | 2.048 | 671.68 | 0.59 | Fruity | | | Al |
| 3 hydroxy 3methylbutan2one | 2.478 | 706.746 | 9.60 | No specific smell | | | P |
| 3 Methylbutan-1-ol | 2.722 | 720.768 | 3.19 | Fruity, Balsamic | A, K, P | A, K | P, Al, A, K |
| 2 Methylbutan-1-ol | 2.771 | 723.499 | 0.57 | Balsamic | A, K | A | A, K, Al |
| Ethyl isobutyrate | 2.986 | 735.792 | 1.07 | Fruity, strawberry | K | A | K, A |
| Isobutyl acetate | 3.175 | 756.59 | 12.93 | Fruity, Banana, pear | K | | K |
| Ethyl 2 methyl butanoate | 3.548 | 779.73 | 0.09 | Fruity strawberry | | | A |
| Ethyl isovalerate | 4.379 | 825.210 | 2.62 | Fruity, Apple | A, P | | A, P |
| Isopentyl acetate | 4.758 | 860.59 | 7.99 | Fruity, Banana | P | A | A, P, K, Al |
| 2methylbutyl acetate | 4.791 | 863.678 | 2.18 | Banana | | A | |
| Isobutyl propionate | 5.408 | 913.13 | 0.16 | Fruity, Banana | | | |
| Camphene | 6.087 | 952.246 | 0.22 | Fruity, camphor | | | A, K, P, Al |
| Isobutyl isovalerate | 7.010 | 1005.623 | 0.10 | Fruity, Apple, raspberry | | | |
| Butyl isobutyrate | 7.104 | 1011.217 | 0.06 | Mixed fruit | | | |
| Caryophyllene | 13.407 | 1431.481 | 0.07 | Fruity, spicy | A, P | | A, Al, K, P |

Abbrevations: RRI=Relative retention index, A=attractant, P=pheromone, Al=allomone, K=kairomone

isopentyl acetate, isopentyl acetate, 2 methyl 1 butanol, ethyl isobutyrate, isopentyl acetate, 2 methyl butyl acetate previously reported as Nitidulidae attractants (22) were found from the flowers of *G. wynaadensis.* Among these compounds isobutanol and 3 methyl 1 butanol are the kairomones of Nitidulidae. (18). The strawberry sap beetle (*Stelidota germinata* (say) (Nitidulidae)), the pest of berries, is attracted to the strawberry odour. 2 methyl butyl acetate was found to evoke an antennal response in female strawberry sap beetle. Curculionidae attractants such as 1Butanol, 3methyl 1 butanol, 2 methyl 1 butanol, ethyl isovalerate, caryophyllene were also detected from *G. wynaadensis* flowers. 1 butanol and 3 methyl butanol attract *Sitophilus granarius* (Curculionidae) (22). Through electroantennography, the response of the antenna to the insect's brain for a given odour can be detected. Ethyl butyrate, Ethyl isobutyrate and Isobutyl propionate are the electroantennogram detection active compounds of Curculionidae African palm weevil, *Rhynchoporus phoenicis* L (23). Methyl acetate, 1 butanol, ethyl butyrate, isobutanol, 3methyl 1 butanol, 2 methyl 1 butanol, ethyl isobutyrate, ethyl 2 methyl butanoate, ethyl isovalerate, isopentyl acetate, camphene and caryophyllene reported as Coleoptera attractants were also identified by GC MS analysis. A mixture of acetaldehyde, ethanol, ethyl acetate, isobutanol, isoamyl alcohol and 2 methyl butanol is commercially available as *Carpophilus* co-attractant (24). Among these compounds, isoamyl alcohol and 2 methyl butanol were found in the GC MS analysis of *G. wynaadensis* flower.

## Conclusion

The present study revealed *G. wynaadensis* anthesis period is only 28hours long. The trapping of pollinators includes synchronising pollinator circadian rhythms with the movement of the petals. The plant is effectively pollinated by *Carpophilus* sp., *Urophorus* sp, *Colopterus* sp of Nitidulidae and an unidentified species of Curculionidae. The assessment of floral scents revealed a number of compounds that serve as semiochemicals for beetle pollinators. Based on the study, the floral aroma of *G. wynaadensis* is dominated by ethyl butyrate, isobutyl acetate, 3-hydroxy-3-methyl-2 butanol, isopentyl acetate, and 3-methyl-1 butanol.

## Acknowledgements

We thank the Director JNTBGRI, Palode, Kerala for providing necessary facilities. We wish to put on record our special thanks for Dr. K. B. Rameshkumar for providing instrumentation facility. This research was supported financially by Kerala University.

## Authors' contributions

AJ and SAK conceived the idea and planned experiments. AJ performed the experiments under the supervision of SAK. All authors analysed the research data and drafted the manuscript.

## Compliance with ethical standards

**Conflict of interest**: Authors do not have any conflict of interests to declare.

**Ethical issues**: None.

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