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Floral Morphology and Reproductive Biological Studies on *Gliricidia sepium* (Jacq.) Stued

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Abstract
The present work aimed to study the floral morphology and the reproductive biology of *Gliricidia sepium* (Family- Fabaceae) at Nasik city, Maharashtra, during the period from the year 2020 to 2024. The flowers of *G.sepium* attract honey bees like Apis spp.; hence it is an important species for honey production. As a result of the colonizing nature of G. septum, it is under no threat at the species level, being found throughout the world especially meso-America and several other countries. Flowers of *G.sepium* found to be bisexual, five petalled with standard petal that is reflexed to 180° at the time of opening which is its characteristic feature. Low rates of fruiting were observed in natural conditions, which suggests that pollination might be failed, probably due to the significantly fewer visits by pollinating agents; establishing the *G.sepium* as a cross-pollination species. Though *G.sepium* has been reported as an insect pollinated species in earlier works; it has been observed that; the flowers have been visited by few insects only; which may be due to the papilionaceous corolla, arrangement of standard and keel petals which tend to protect the stigma and keep it enclosed; posing difficulty for insects in gaining access to the nectaries.

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Introduction

Gliricidia sepium (Jacq.) Steud. Belongs to family Fabaceae and is native to south and Central America. It has wide distribution in most of the tropical and sub-tropical regions as a shade ornamental tree. Being an aggressive pioneer species, *G.sepium* can be found in a wide range of habitats; varied environments and climatic conditions. (Lavin; 1996) [14]. Also, it is used for many other purposes like green manure and firewood crop. (Sumberg, 1985; Aluri and Reddi; 1996) [1, 2]. In India; it has been cultivated under social and community forest programs. Plants have a broad range of floral traits floral diversity is associated with adaptation to different specialist pollinators (Johnson, 1996) [3]. Also, floral diversity has to be appreciated as the result of pollinator mediated selection in plants. Moreover, floral characters can directly influence pollinator behavior and consequently affect plant reproductive success, either increasing pollinator visit frequency or pollination quality. (Pang et. al; 2012) [4].
G.sepium is small to medium-sized tree, found to be cultivated as a fast- growing species having high capacity for regeneration, resistance to drought and facility to propagate sexually and asexually. Moreover, it has been exploited as forage for its high nutritional value as well as an energy alternative (Drumond and Carvalho Filho, 1999; Kiill and Drumond; 2001) [11, 12]. Currently, most studies on *G.sepium* mainly focus on its use as natural fertilizers to improve soil fertility (Aroonluk and Talubnak *et al*.; 2020) [5], its role of atmospheric nitrogen fixation in agroforestry.

Its impact on intercropping system (Beedy *et al*. 2010) [8], its bio efficacy in agriculture systems against rodents like rats (Tariq *et al*; 2016) [7] and for improving soil fertility (Kumar and Mishra; 2013) [9], review of *G.sepium* with respect to its pharmacological properties (Jasmine and Sundaram *et al* 2017) [10]. However, the floral biology of *G.sepium* emphasizing the floral morphology along with phenological are crucial aspects which influence its reproductive success; have rarely been investigated.

Methodology

Study Site: The study was conducted during the period from the year 2019 to 2020 at the foothills of Chamar leni, located northeast of Nasik city, Maharashtra. (Lat N 19° 58'32.18736" Long E 73° 49' 59.71404"). The Nasik city has Nasik is prominently represented by the eastwardly flowing the "Godavari river" with its tributaries; and Western Ghat region with peaks like Kalsubai (1646 meters) and Trimbak (1294 meters). Though the city has been popularly known as Grape city or wine city and leading onion producer of Maharashtra; the vegetation of Nasik city is mainly composed of some rare, medicinally important tree species like *Cochlospermum religiosum*, *Bombax ceiba* L. *Butea monosperma* (Lamk) Tauub. *Erythrina variegata* L. *Couroupita guianensis*, *Neolamarckia cadamba* (Roxb.) Bosser etc. The city has tropical wet and dry climate with the temperature rise slightly in the month of October, followed by the cool season from November to February, with an average temperature of around 28°C during the day and 10°C in the night.

Floral Morphology

For investigation of the floral morphology 30 flowers from 10 inflorescences/flowering twigs were selected at random, and were studied fresh at site with a hand lens (60X increase); after which brought in the laboratory, stored in 70% ethanol. The floral parameters like lengths of floral bracts, petals, stigma style etc. were measured using digital caliper and a stereomicroscope (Leica M80). Also, other floral reproductive attributes like stamens, pollen grains and ovary, fruits, seeds etc. were observed so as to assess reproductive behavior.

Phenological aspects

During the field visits at the study area; trees of *Gliricidia sepium*; with an average growth were selected and marked. Each tree was monitored once in a fortnight so as to record the phenological events such as beginning of leaf fall, the commencement of leaf initiation, initiation of budding and flowering, anthesis, end of flowering, beginning of fruiting, completion of fruiting and fruit dispersal. (Prasad & Hegde 1986; Arunkumar and Chandrasekhar *et al*. 2020) [16, 17].

Floral visitors

For observation of floral visitors; monitoring of inflorescences was done between 7.00 hrs to 11.00 hrs during daytime and 18.00 hrs to 22.00 hrs during night time for weekly duration by separate observers of total 60 hrs. of monitoring. Simultaneously visitors were photographed and captured with insect nets so as to identify correctly with the help of available literature and experts. Night observations were undertaken using a flashlight.

Pollen grain studies

Pollen output per flower was calculated by counting the number of pollen grains per anther and multiplying it with the number of anthers per flower. Anthers are separated from excised flowers which were kept at room temperature. Pollen grains are then collected on slide after the dehiscence of anthers. Morphology of pollen grains was studied and Pollen viability is determined by acetocarmine staining test (Shivanna *et al*; 1991, Shivanna and Rangaswamy; 1993) [25, 26].

Results and Discussion

Floral Morphology

In *Gliricidia sepium* flowering appears generally during dry seasons when trees are partially or totally leafless. Flowers found to be borne on axillary inflorescences, raceme type, with centripetal development. The flowers are bisexual, five petalled with a standard petal that is reflexed to 180° at the time of opening which is its characteristic feature. Also, there are the cup-shaped calyces which persist well into fruit maturation. *Gliricidia sepium* can be distinguished due to its conspicuous, pink colored inflorescences (length ranges from 2.5 to 15-17 cm) arranged in upward curving manner. The individual flowers borne on a well-developed hypanthium and with a smooth stalks and calyces. Calyx is glabrous with inconspicuous five more-or-less evenly spaced lobes. The corolla has pink colored petals with a predominant magenta color and a median portion of the cream-colored banner that acts as a nectar guide. Typically, inside each flower; a nectar guide; more or less equal to the length of keel petals, deep pink to slightly pink in color with its blade that can be reflexed about 180° at anthesis; is present. Keel petals are pinkish in color, 15-22 mm, long straight with blunt tips. Stamens are diadelphous, with anthers attached basally to the filaments. Pollen grains are spheroidal, tricolate, Polar Regions smooth. Ovary is with stipe as long as the calyx tube, consists of 7-12 ovules. Style is glabrous, 4-5 mm long at anthesis and with a terminal stigma. The pods contain 3-10 seeds each and are explosively dehiscent, laterally compressed and pale green or reddish-green when unripe, turning yellow-brown when fully ripe. (Lavin and Sousa, 1995; Lavin; 1996) [13, 14].

Phenological aspects

Gliricidia sepium is a deciduous tree which shed its leaves well before the flowering period starts; i.e. it flowers in the dry season once it has shed its leaves. The reduction of soil moisture and atmospheric humidity might be providing a stimulus to flowering in *G.sepium* and other species from tropical regions. (Kiill and Drumond; 2001) [12]. Defoliations start in the month of late January and completed up to middle or end of the February. As soon as defoliation starts; an initiation of flowering begins with appearance of floral buds on woody stems i.e. flowering period range from late January and full flowering was observed during the mid/late February. The leaflets; 5 to 25 in number, have tapering and pointed apices at their maturity; and simultaneously the pods start to develop fully. Within the selected trees; there is no strict synchronicity was observed in flowering and fruiting; however; individual trees were observed to reach peak flowering with an interval of 15-40 days. Hughes (1987) [19]. Anthesis was observed daytime early in the morning;

reaching maximum between 7.30 hrs to 8.30 hrs. (Marak and Wani: 2018) [23]. The flowering time period was found around 10 hrs after which floral senescence occurs which is characterized by withering and the color change of the banner.

Floral visitors

Though *G.sepium* has been reported as an insect pollinated species in earlier works; it has been observed that; the flowers have been visited by few insects only; which may be due to the papilionaceous corolla, arrangement of standard and keel petals which tend to protect the stigma and keep it enclosed; posing difficulty for insects in gaining access to the nectaries. However, few species of bees, moths, ants and thrips have been observed as floral visitors during the present study. These insects can insert their proboscis into a small opening so as to get the nectar without having a role in pollination; as they do not come into contact with the stigma or the anthers. These observations agree with the findings made earlier works of Frankie *et al*., 1976; Simons; 1996 [18, 15]. The flowers of *G.sepium* attract honey bees like Apis spp.; hence it is an important species for honey production (Katende, 1995) [27]. On the basis of observed floral visitors; bees were found to be most frequent. (Khan and Verma *et al*. 2021) [24]. Among bees; mostly the Apis mellifera and Xylocopa sps. Have been found as floral visitors more frequently during the daytime; especially 1-2 days after flower opening. (Janzen; 1983; Anandhan *et al*.; 2020) [22, 21]. The frequency of floral visitors has been varied significantly probably due to the density of *G.sepium* at study site, availability of alternate nectar sources, abundance of predators and parasites etc.

Pollen grain studies

Pollen grains of *G.sepium* were found yellowish in colour, spheroidal and tricolate with an average size recorded as 0.40µm. The pollen viability was determined by acetocarmine test at room temperature (Shivanna *et al*.; 1991) [25] revealed that pollen grains of *G.sepium* are highly viable i.e. 79.93% at the first day of their collection; However, this viability goes on decreasing during successive weeks i.e. 43.43% & 21.3% at the 7th and 15th day of pollen collection respectively. (Table-1). These values are in accordance with the similar findings made by Kanthaswamy; 2006, Wani and Chauhan; 2009, Marak and Wani; 2018 [30, 29, 28].

Studies of phenology and reproductive biology are important for conservation and improvement programs. The number of flowers and seeds borne by trees also affects their contribution towards the pollen and seed genetic pools. This has implications for sampling variation and also for the prospect of genetic improvement. As a result of the colonizing nature of *G.sepium*, it is under no threat at the species level, being found throughout meso-America and several other countries. Low rates of fruiting were observed in natural conditions, which suggests that pollination might be failed, probably due to the significantly fewer visits by pollinating agents; establishing the *G.sepium* as a cross pollination species. (Aken'ova and Sumberg; 1986, Aluri and Reddi; 1996, Anandhan *et al*.; 2020) [20, 2, 21].

Table 1: Pollen grain viability by acetocarmine test for three different trees of *G. sepium*

No. of trees /Days of Pollen collection	T1	T2	T3	Mean
1st day	81.50	79.40	78.88	79.93
7th day	46.20	42.80	41.30	43.43
15th day	22.60	21.50	19.80	21.3

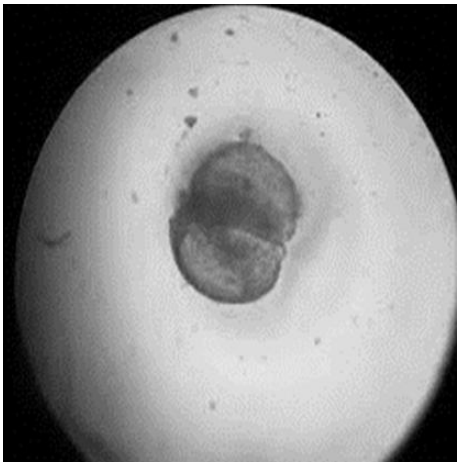

Fig 1: Pollen morphology

Fig 2: Pollen germination

Fig 3: *Gliricidia sepium*

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