REPRODUCTIVE BIOLOGY OF WATER LILY

(*Nymphaea nouchali* Burm.f.)

By

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(2010-11-107)

THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Reproductive biology of water lily (*Nymphaea nouchali* Burm.f.)"** is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Introduction

1. INTRODUCTION

Water lilies are a group of fascinating aquatic rhizomatous perennial herbs belonging to the genus *Nymphaea*. Among the different species of *Nymphaea*, *Nymphaea nouchali* is the one common in South India. This species with long petioled leaves and lamina floating on the water surface is commonly known as the blue water lily, blue star water lily or star lotus. This species which forms an important constituent of aquatic flora possesses immense ornamental and medicinal values.

Blue star water lily is widely used as an ornamental plant due to its beautiful and spectacular flowers. The plant has been long valued as a garden flower in many countries including India, Thailand and Myanmar for giving aesthetic view to the ponds and gardens. Many flower colour variants like violet, blue, white, pink etc. are available in this species. It is also popular as an aquarium plant under the name 'dwarf lily'. Flowers are widely used for decorative purposes. Cut flowers stand transportation if picked in the mature bud stage.

Water lily flower is the national flower of Bangladesh and Srilanka. The plant is historically and functionally significant since it is associated with our culture and tradition. There are several mythological stories associated with this beautiful aquatic flower. The flowers are used for religious purposes also. In Buddhist mythology, it is claimed that this flower was one of the 108 auspicious signs found on prince Siddhartha's footprint.

Nymphaea nouchali Burm.f. is an important and well known medicinal plant as well. The rhizomes, flowers and seeds form an important ingredient of many ayurvedic formulations and siddha medicines for the treatment of diabetes, inflammation and urinary disorders. In Ayurveda, under the name 'ambal' the rhizomes are used in the treatment of irritable bowel syndrome, sore throat and bronchial congestion. The various parts of this plant are used by traditional healers for treating various diseases. The flowers of water lily are having a

sedative effect which makes them useful in the treatment of insomnia and nervous disorders. The hypoglycemic and hepatoprotective effects of rhizomes have also been confirmed.

The rhizomes, either boiled or roasted can be consumed. The tender leaves and peduncles are also valued as food. The dried plant, collected from ponds, tanks etc. during dry season is used as animal forage.

Despite its immense potentialities water lily has received only very little attention of crop improvement workers. By adopting proper breeding techniques plants of high economic value can be produced. Improvement with respect to economic and aesthetic aspect is of great significance in water lilies. Prior to genetic improvement study on taxonomy, morphology and reproductive biology are demanding. A thorough knowledge of the reproductive biology which includes anther dehiscence, stigma receptivity etc. and the developmental pattern of flowers and fruits is fundamental to an understanding of the dynamics of natural population. These informations are essential for the success of any crop improvement programme particularly those involving hybridisation. Besides, palynological features have great relevance to our understanding of the systematic relationships among the different species of *Nymphaea.* Being a crop of tremendous potential the present investigation was undertaken with the following objectives.

1. To elucidate the reproductive biology of *Nymphaea nouchali.*
2. To evaluate the developmental pattern of flowers and fruits in *Nymphaea nouchali.*

Review of Literature

2. REVIEW OF LITERATURE

The genus *Nymphaea* commonly known as water lilies is one of the few fascinating group of aquatic plants which are giving aesthetic view to the Indian waters because of its beautifully coloured flowers.

Water lilies in broad sense include the purely aquatic families like Nymphaeaceae, Nelumbonaceae, Cabombaceae and Barclayceae. Among these, Nymphaeaceae appears to be the most complicated group owing to its diversity and intraspecific variations especially in the flower colour in different populations. The other families are represented by one or two definite species. In India, the family Nymphaeaceae is represented by two indigenous genera *Euryale* and *Nymphaea* in addition to the Amazon Giant water lily belonging to the genus *Victoria*. Among these three genera, the genus *Nymphaea* remains complicated and confusing in the absence of a consolidated taxonomic revision, while the plants of monotypic genus *Euryale* and exotic genus *Victoria* are easily recognizable due to their well defined morphogenic features (Ansari and Jeeja., 2009).

Chukiatman (2006) described that the plants in the family Nymphaeaceae are the oldest group of plants on the earth. Recently the botanists have split this family into two. Those with rigid petiole and peduncle are grouped under Nelumbonaceae. Others with soft petiole and peduncle are grouped under Nymphaeaceae.

Friis *et al.*,(2005) reported that Nymphaeaceae is a primitive family and the fossil record goes back to the early cretaceous period. Nymphaeaceae is classified under the order Nymphaeales in the group of the "basal families", in the recent molecular based angiosperm phylogony (Judd *et al.*,2002; Anonymous, 2003).

As reported by Woods *et al.*,(2005), *Nymphaea* is the most diverse genus in the family Nymphaceae containing over 50 species distributed in the tropical and temperate regions.

Mabberley (1997) reported that the family Nymphaeaceae is cosmopolitan with about 6 genera and 75 species. Gandolfo *et al.,* (2004) described that Nymphaeales comprise 8 genera and approximately 70 species of aquatic plants with a world wide distribution in tropical and temperate region. They can be seen in luxuriant growth inhabiting ponds, lakes and quiet back waters (Everett, 1981).

Genus *Nymphaea* consists of spectacular water plants with floating leaves and large attractive flowers. *Nymphaea stellata* and *Nymphaea rubra* are native to India. With their numerous varieties and hybrids, having exquisite blooms of various colours and attractive foliage, the water lilies are considered as "Table Jewels of Water garden" (Muthukulam, 2006).

Nymphaea nouchali Burman f. (Syn. *Nymphaea stellata* Willd.) belongs to the family Nymphaeaceae. 'Nymphala' in Greek refers to water nymph and 'stellata' in Latin means star shaped (Wiart, 2006). For a variety of reasons a lot of synonyms occur for *Nymphaea stellata* (Danin, 2000 and Merlin 2003). The synonyms are *Nymphaea malabarica* Prior., *Nymphaea minima* F.M. Barley, *Nymphaea punctata* Edgew, and *Nymphaea versicolor* Sims (Stephens and Dowling, 2002). *Nymphaea caerula* is also considered as a synonym by some botanist, while some include a variety *Nymhaea nouchali* Burm.f. var. caerulea (Savigny) Verdc. (Viljoen and Notten, 2002). Other varieties recorded are *N.nouchali* var. cyanea (Hooker F. and Thomson), (syn. *N.stellata* var. cyanea), and *N.nouchali* var.versicolor (Roxburgh) Hooker f. and Thomson (syn. *N. stellata* var.vesicolor) (Slocum, 2005). *N. nouchali* is the synonym for *N. stellata* (Verdcourt,1989; Simmonds and Howes, 2006).

However in some literature *N. nouchali* and *N. stellata* have been differentiated into two species (Singh and Sandhu, 2003). Till date, there exists a controversy among botanists regarding the synonyms and the varieties.

Slocum (2005) observed that *N. stellata* is a perennial aquatic rooting herb, wild or cultivated, generally found in tanks and ponds throughout the warmer parts of India, particularly the Eastern Ghats. For centuries it has been

cultivated in Southeast Asia, especially around temples. Raja *et al.,* (2010) reported that it is distributed in Philippines, Borneo, Srilanka, Myanmar, Afganisthan, Pakistan, Bangladesh, Nepal, Cambodia, Malaysia, Laos, Thailand, Vietnam, New Guinea, Indochina to China, Taiwan, Indonasia, Africa and Australia.

Nymphae nouchali is commonly known as Indian blue water lily or Indian water lily in English and has different vernacular names (Appendix I). As reported by Slocum (2005), water lily is often referred as 'blue lotus of India' but it is not a lotus. The name 'Neelathamara' is applicable only to the water lily with bluish flowers, which is *Nymphaea stellata.* Many reports specify that 'blue lotus of the nile' and 'blue lotus of India' are *N. caerulea* and *N. nouchali* respectively, while others report 'sacred blue lily' as *Nymphaea nouchali* var. caerulea.

Henry (2008) reported that water lilies are the most exquisite and colourful plants in the water garden which can give months of pleasure during the summer. Water lilies are either day blooming or night blooming.

The Indian system of medicines, particularly Ayurveda and Siddha, uses *N. stellata* as a single drug or in combination with other drugs. *N. stellata* is an ingredient of many ayurvedic formulations like Ashokarishta, Arvindasava, Usirasava, Candanasava, Kalyanaka ghrita, Samangadi curna, Kanaka thaila, Jatyadi taila, Manjesthadi thaila, Candanadi lauha and Triphala ghrita (Anonymous, 2001).

The flowers of *Nypmphaea nouchali* Burm. have a wide range of applications in Ayurveda and traditional medicine. Studies have been taken up by Nagavani and Rao (2010) to evaluate the enzymatic, non enzymatic and antioxidant potential of ethanol, methanol and aqueous extracts of fresh and dry flowers from *N. nouchali.* Results revealed that the levels of non-enzymatic antioxidants like phenols, flavinoids, tannins etc. as well as potential of antioxidants were found to be more in methanol extracts of *N. nouchali.* The

levels of enzymatic antioxidants were found to be high in aqueous extracts from fresh flowers of *N. nouchali.*

It is also an ingredient of many polyherbal formulations for anti-aging, rejuvenation and menstrual irregularities. The traditional uses of *N. nouchali* are given in Appendix II. The rhizome, fruit, leaf petiole, roots, flowers, tubers and seed are used as edible parts in different ways by people (Raja *et al.,* 2010).

Slocum (2005) reported that *N. nouchali* has also been cultivated for food in Srilanka and rhizomes are full of starch and quite tasty when boiled. The roots and rhizomes are considered to be nutritious when eaten either raw or roasted. Flower and flower stalks are used as vegetables, green manure and fodder.

In a study conducted by Babu *et al.,* (2009), different solvent extracts of entire plant have shown the presence of sterols, alkaloids, saponins, tannins and flavonoids. Nymphayol (25,26-dinorcholest-5-en-3b-ol), a new sterol has been from successive chloroform extract of the flower. Protein, pentosan, mucilage and tannins are reported in the seeds by Kapoor *et al.,* (1975). Astragalin, corilagin, gallic acid, gallic acid methyl ester, isokaemferide, kaempferol, quercetin-3-methyl ether, quercetin, 2,3,4,6-tetra-o-galloyl dextroglucose, and 3-o-methyl quercetin-3'-o-beta dextroxylopyranoside have been identified in flowers by Kizu (2003).

Raja *et al.,* (2010) reviewed the antidiabetic activity, tumour inhibition property, antihepatotoxic, analgesic, anti-inflammatory and antimicrobial activities of *N. nouchali.*

Heavy metal accumulation studies in Nymphaea were carried out from eight different locations along the stream using the atomic absorption spectrophotometric technique by Shuaibu and Nasiru (2011). The results showed that the plant has high tendency to selectively bioaccumulate zinc and lead suggesting that it could be used to monitor zinc and lead levels in the stream. The

study also indicated that the plant has high potential for selectively uptaking lead and zinc faster than cadmium and iron in the stream.

Free radical scavenging activity of hydroalcoholic extracts of dried flowers of *Nymphaea stellata* Willd. was reported by Rakesh *et al.*,(2010).

Karyotype analysis on *N.stellata* showed 2n=28 (Ping-he and Wei-pei, 1994). Another study by Hossain *et al.*, (2007) on chromosome number of *N.nouchali* revealed euploidy in this species. Three types have been identified, *N.nouchali* (Type 1) 2n=56, *N.nouchali* (Type 2) 2n=84, and *N.nouchali* (Type 3) 2n=70. *N.nouchali* (Type 1) 2n=56 (4x) chromosomes may have evolved by the doubling of the chromosome (2n=28) from the ancestral species. Similarly *N.nouchali* (Type 2) 2n=84 (6x) may have evolved from the chromosome doubling of *N. daubeniana* 2n=42 (3x). *N.nouchali* (Type 3) 2n=70 (5x) might have originated by the crossing of *N. pubescence* 2n=84 (6x) and *N.nouchali* (Type 1) 2n=56 (4x). Most of species in *Nymphaea* hybridize freely among themselves naturally, and thereby, generate uncertainty regarding their identity.

Genotypic studies by Albert *et al.*, (1992) revealed that carnivory is polyphyletic. Phylogenic trees prepared on the basis of taxonomy suggest a strong evolutionary linkage between some carnivorous families such as *Nepanthaceae* and *Sarraceniaceae* to *Nympheaceae*. This is conclusive from *N. stellata* as it indulges in primitive form of insectivory. No insectivorous flowering plant has been reported and *N.nochali* may be the missing link in the evolutionary history of other highly evolved carnivorous plant families (Tetali *et al.*, 2008).

`Improvement with respect to economic and aesthetic aspect is of great significance in water lilies. Prior to genetic improvement of *N. nouchali*, study on morphology and reproductive biology are demanding.

2.1. LEAF CHARACTERS

Decorative water lilies add beauty to the pool. The shade formed by the leaves is useful for reducing the luxuriant growth of algae and provide shelter to fish (Brickell, 1989). Rossow and Charboneau (2006) reported that water lilies grow at the base of the pond and produce leaves and blooms to the surface. They also provide valuable leaf cover, which reduces algal growth.

Dassanayake (1996) described the leaves of *N.nouchali* as glossy bright green on the upper surface and dark purplish green beneath. The leaves are glabrous on both surfaces with green prominent veins on the lower surface. The petiole is terete and purplish green in colour.

Grob *et al.* (2006) observed that in *Nymphaea prolifera* the leaves arise from the rooted tuber consisting of petioles of up to 120 cm long. Hulten (1968) reported that the leaves of *Nymphaea tetragona* are elliptic-oval and is up to five inches long.

Wooten (1986) found that many floating leaved plants have increasingly longer petioles with increasing water depth. In *Nymphaea odorata* the petiole length varies according to the water depth (Dalton and Novelo, 1983). In *Nympheae odorata* the growth of aerial petiole may be an adoptive response to shading, allowing the aerial leaves to raise above the crowded water surface (Etnier and Villani, 2007).

Kunii and Aramaki, 1992, reported that mean final potential leaf area peaked in early July (146 cm^2) and then decreased. In *Nymphaea tetragona* the leaf life span reached an initial peak in June (38.6 days) and gradually decreased to minimum (20.5 days) in late August and then increased suddenly to a maximum (52.9 days) in October. Annual mean life span was 31 days. As suggested by Chabot and Hicks (1982), many factors relate to the leaf life span and causes of decomposition can be recognized in the form of damage types and infection or decomposition patterns.

2.2. FLOWERING BIOLOGY

2.2.1. Growth Pattern of Flower Bud

Krishnan *et al.*,(2004) observed that the general phenomenon that the night blooming flowers are white in colour does not seems to be true in water lilies.

The circadian rhythm of flower opening is seen in water lilies.

Studies on three *Nymphaea* species, *N. alba*, *N. rubra*, and *N. stellata* by Deviprasad (2009) revealed that *N. stellata* was superior to other two in all the vegetative parameters, except leaf length and longevity which were higher in *Nymphaea rubra*. Early flowering and maximum flower production were also observed in *Nymphaea stellata* in which duration from bud emergence to complete flower opening was minimum. Length of bud, flower size and longevity of flowers were found to be maximum in *Nymphaea rubra*.

Astle (2006) reported that water lilies require full sunlight as the development of bud is dependent on amount of sunlight. Day length and temperature are two main factors influencing the flower production of hardy water lilies when grown in tropics (Songpanich, 2007). Factors changing during the whole day regulate the amplitude of opening of the flower of *Nymphaea* and its immersion. The intensity of the bloom of *Nymphaea* depends up on the temperature of the air and the water (Volkova *et al.*, 2001).

Solcum (2005) found that some *Nympheae* species do not bloom during the period of high temperature.

2.2.2. Floral Morphology

Detailed morphological studies have been carried out in family Nymphaeaceae (Khanna, 1967).

Rodriguez (2007) described that there are two types of water lilies, tropical and hardy. Tropical water lilies are divided into day and night bloomers. Hardy

water lilies are all day bloomers and some of the hardy water lily flowers change colour over the life of the bloom.

Soltis and Soltis (2004) observed that the floral characters are varying extensively among the genera, ranging from small, simple monocot like flowers of *Cabomba* to very large, showy, elaborate flowers of *Nymphaea* and *Victoria.*

Songpanich (2007) in his experiment on 'Flowering Habits of Hardy Water Lilies in the Tropics' revealed that the maximum number of flowers produced by *Nymphaea* (Gloriosa) was 163 blooms per year and minimum number of flowers by *Nympheae* (Perry's Fire Opal) was 28 blooms per year.

The flowers of *Nymphaea rubra* are large, double red in colour and they open in night during summer (Muthukulam, 2006) and are sweet scented, large, 4-10 inches across (Biswas and Calder, 1984).

Muthukulam (2006) reported that *Nymphaea stellata* flowers are medium to large in size and pale blue in colour. Jokla and Mussob (2000) observed that *Nymphaea alba* is a white flowering species with yellow stigmatic disk. The four sepals are lanceolate as well as 15-30 petals, which gradually turns into stamens. There is a gradation in outward direction from conventional stamens to staminoides and petals. Dalton and Novelo (1983) found that the flowers of *Nymphaea odorata* are up to 6 inch wide.

Schneider (1982) in his experiments on *Nymphaea elegans* observed that flowers open each morning for three consecutive days. *Nymphaea odorata* buds open in early morning exposing the spectacular white, waxy flowers which float on the surface of water. Some petals close during afternoon and some petals remain open (Dalton and Novelo, 1983). In *Nymphaea prolifera* the rooted tubers produce mother flowers with flower stalks up to 80 cm long, but the stalks of daughter flowers are usually less than 20 cm long (Grob *et al.*, 2006).

Studies in *N. stellata* by Biswas and Calder (1984) showed that it has four sepals which are narrow with fine purple lines, eight petals and 15-29 stamens.

Apices of the anthers are foliacious with tongue shaped appendages and an eight rayed stigma.

Wiersema (1987) observed that in *Nymphaea* the bractless flowers have mostly tetramerous calyces, whereas the other floral organs occur in whorls and rings. The petals occur in various shades of white, blue, red or yellow.

The flowers of *Nymphaea gigantea* growing in Western Australia bloomed on at least 4 consecutive days, opening by 09.30 h and closing by 17.00 h. The first day flowers were 20 cm above the water, inodorous and with a dark purple-violet corolla, which, with the calyx, formed a funnel-shaped perianth. On succeeding days the flowers became raised to 30 cm above the water, and the corolla faded to light purple-violet. The flowers were then functionally staminate and the stigmatic surface was dry and non-receptive (Schneider, 1982).

Dassanayake (1996) described petals of *N. nouchali* as pale violet or pale blue fading to a dull blue and yellowish at the base and is lanceolate or narrowly elliptic in shape. The flower opens from sunrise to early afternoon.

As reported by Dalton and Novelo (1983), the flowers of *Nymphaea odorata* are white in colour with four sepals, 56-100 stamens and up to 20 pistils.

The anthesis in *N.capensis* was observed to be with flowers opening and closing for three consecutive days. (Orban and Bouharmont, 1995).

A study conducted by Begum *et al.,*(2010) on morphology and floral biology of three species of the genus Nymphaeaceae of Bangladesh revealed that *N. nouchali, N.pubescence* and *N.rubra,* were normal, protogynous or normal and protogynous, respectively and *N.nouchali* flowers opened for three consecutive days.

Opening and closing time of flowers were very much influenced by the intensity of sunlight and hence temperature also. According to Prance and Anderson (1976), temperature was more effective than sunlight for the opening and closing of *Nymphaea* flowers.

The *N.alba* and *N.candida* studied by Velde and Vander (1986) were day-flowering only, whereas *Nuphar lutea* flowered during the day and night without any closing movement of flower parts during anthesis.

Flowers in *N. lotus* open at sunset and close in the morning hours, thereby revealing a high variability in timing (Hirthe and Porembski, 2003).

2.3. DETERMINATION OF ANTHER DEHISCENCE

Begum *et al* (2010) reported that the behavior of anther dehiscence and stigma appendages in genus *Nymhaea* had significant role in pollination. Excellent pollen germination and receptivity were observed in the exudates of stigma cup.

Capperino and Schneider (1985) based on studies on *N. mexicana* found that anthesis is diurnal with flowers opening and closing for two consecutive days. Flowers remain functionally female on the first day of anthesis.

Wiersema (1987) reported that the pollen release takes place on the second day of flowering.

In *Nymphaea capensis* the outermost anthers are dehiscent on the first day, and middle anthers on the second day of flowering (Orban and Bouharmont, 1995).

Specific differences in the duration of anthesis in the species studied by Velde and Vander (1986) appeared to be due to variation in ripening of the rows of anthers and could be related to the considerable differences in the number of stamens in individual flowers. The male phase of flowering was prolonged in all species.

Zhou and Fu, 2007 noticed that anthesis in flowers of *Nuphar pumila* occurs for four consecutive days and flowers open completely on the first day of anthesis.

Anthesis show great variation in among different species of *Nymphaea* which was collected from Bangladesh and in *N.nouchali,* it was for three consecutive days and anther of different whorls burst gradually from outermost to innermost whorl during successive anthesis. In *N. nouchali* the anther lobes opened for ever but in *N. rubra* and *N. pubescens* opening and closing correspond to the spreading of petals. The anthers were introse and split longitudinally in all the species (Begum *et al.*, 2010).

2.4. DETERMINATION OF STIGMA RECEPTIVITY

Schneider (1982) observed that in *Nymphaea elegans* on the first day, the flowers are protogynous with stigma secreting a fluid which fills a perigynous cup. During second and third days of flowering the flowers are functionally staminate.

Wiersema (1988) reported that sexual reproductive strategies in genus Nymphaea is mostly protogynous.

The studies on *Nymphaea alba* L., *Nymphaea candida* Presl, *Nuphar lutea* (L.) Sm.) by Velde and Vander (1986) suggested that all the three species are protogynous.

Orban and Bouharmont, (1995) reported that flowers are homogynous in *N. capensis* and gynoecium is characterized by wet-papillate stigma, a short hollow style and secretory cells on the ventral surface of the ovary.

The stigmatic cup holds 2.2-3 ml of a watery fluid which is acedic (pH 4-4.5) in nature. The rim of cup is surrounded by a row of 3mm long clavate shaped appendages, curved inwards, which are smooth and glabrous and produced at the tips of locules (Tetali *et al.,* 2008).

Shivanna and Shastri (1981) observed the correlation between presence of stigma exudates and pollen germination in *Amaryllis vittata* and *Crinum defixum.* According to them the protein (esterase) required for pollen germination was

present in stigma exudates. Horn like small stigma appendages played an important role in breeding behaviour of the species.

The cup-shaped gynoecium at the base was filled with fluid secreted from the stigma in *N. gigantea* (Schneider, 1982).

In the study conducted by Begum *et al.* (2010), pollen grains showed the highest germination percentage on the stigma of the flowers opened for the first time. On the second anthesis *N. rubra* lost its receptivity but *N. nouchali* and *N. pubescence* showed excellent pollen germination. Third anthesis showed negligible stigma receptivity.

Flowers of *N. lotus* are protogynous and anthesis lasts for 4 to 5 days. (Hirthe and Porembski, 2003).

2.5. POLLINATION BIOLOGY

Capperino and Schneider, (1985) observed the visiting of honeybees and *Dialictus connexus* in *Nymphaea capensis,* moving rapidly from flower to flower and foraging vigorously for pollen on the stamens of second-day flowers. When they landed on the wet stigmatic surfaces of first-day flowers, the fluid washed or loosened pollen from the bee's bodies, thus effecting pollination. Flies and beetles also visited the flowers of *N. capensis.*

Two pollen-foraging bees (*Trigona* sp.) were observed in *Nymphaea gigantea* to slip into the stigmatic fluid, which washed off most of the pollen they carried and the pollen became deposited on the stigmatic surface. In addition to *Trigona* and a single individual of *Leioproctus (Anacolletes)* sp., several beetles actively foraged for pollen, but only *Trigona* came in contact with the stigmatic pool (Schneider, 1982).

Tetali *et al* (2008) recorded six species of insects visiting the flower to collect pollen in *N. nouchali.* The species were confined to Diptera and Hymenoptera, the most common being the two species of honey bees (*Apis florae*

and *Apis mellifera*) and two species of solitary bees. He also described selective insectivorous nature of the flower, where insects are trapped in the stigmatic cup which was not reported in flowering plants before.

Flowers of *N. lotus* were visited by *Ruteloryctes morio*, an endemic dynastid beetle during the night. Beetles copulated in the flowers and fed on flower parts, but were less effective pollinators than several bee species that visited flowers in the early morning. *N. lotus* thus seems to be adapted to pollination by both nocturnal beetles and diurnal bees (Hirthe and Porembski, 2003).

The role of thermogenesis in the pollination biology and production of Amazon Water lily was studied by Seymour and Matthews (2006).

2.6. PALYNOLOGY

Pollen grains of Nymphaeacea are described in literature as monocolpate except in the genus *Nelumbo*. The grains are bilaterally symmetrical and are generally single aperturate in the other five genera of the family viz., *Nymphaea, Brasenia, Cabomba, Eurayle* and *Victoria* (Erdtman, 1952).

Pollen morphology of nine species and six ecotypes of *Nymphaea* was analysed by Ansari *et al.,* (2005) by using LM and SEM. The pollen grains are trimorphous with monocolpate, megaporate and trichotomocolpate forms occurring individually or in combination in different taxa. The exine surface is dominantly verrucate showing different distributional patterns forming areolate to sparsely verrucate, and with modifications of these excrescences, which are connate or surmounted by protruberances. The pollen types are diagnostic at species and ecosystem levels, and appear to be of taxonomic and evolutionary significance in the genus.

Osborn *et al.,* (1991) reported that the main difference between the lotus and water lily is the triaperturate pollen of *Nelumbo* and monoapertunate pollen

grains of most Nymphaeles. In *Nuphar pumila* pollen grains are monosulcate with long spines (Zhou and Fu, 2007).

2.7. FRUIT DEVELOPMENT

Tetali *et al.*,(2008) observed that the flowers in *N. nouchali* remain functionally open for 3-4 days after which the peduncle sinks into the water and develops in to fruit. The fruit is covered with a persistent calyx and ripens under water.

Excellent fruit set and ellipsoid seeds were reported in *N. nouchali* and *N. pubescence* and no natural seed set in *N. rubra* by Begum *et al.* (2010). *Nymphaea caerulea* Savigny do not set fruits in India (Ansari *et al.*, 2005).

Materials and Methods

3. MATERIALS AND METHODS

The investigation entitled 'Reproductive biology of water lily (*Nymphaea nouchali Burm.*f)' was carried out in Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara, during the period from August 2010 to July 2012. The details regarding the experimental materials and methodology adopted for the study are presented below.

A. Materials

Two flower colour variants of *Nymphaea nouchali*, blue and white types, collected from Malabar Botanical Garden, Calicut, and maintained in cement tanks in the Department of Plant Breeding and Genetics were selected for the study. Clay and water level were retained at uniform height in all the tanks throughout the experimental period. The developmental pattern of flowers as well as flowering biology were critically evaluated in the selected types under *ex situ* condition.

B. Methodology

3.1. LEAF CHARACTERS

The morphological features of leaves from both the colour variants were described by observing fully mature leaves. Observations on various biometric characters of the leaf like the length of petiole, length of lamina, length of notch, width of lamina at the base, middle and tip were taken from ten fully developed leaves of each colour variant.

3.2. FLOWERING BIOLOGY

3.2.1. Growth pattern of flower bud

Ten flower buds each from the two colour variants were tagged immediately after their appearance on the surface of the mud. The growth of flower bud from visual appearance stage till opening was studied at periodic intervals in both colour variants. The days taken by the flower bud from initiation

to reach the water surface as well as till opening were noted. The pedicel length, rate of elongation of pedicel, size of fully mature flower bud as indicated by its length and circumference, diameter of fully opened flower and longevity of flower in both types were also observed. The process of opening and closing of the flower was thoroughly examined in both types.

3.2.2. Periodicity of flowering

The number of flowers produced in each month in both the colour variants was recorded and expressed as percentage of the total number of flowers produced per year. The seasonality of the flowering was then computed. For the convenience of analysing the seasonal effect on flowering, the whole year was divided into four seasons, December-February (winter), March-May (summer), June-August (rainy season) and September-November (spring). The succession of flower formation in peak flowering period was also observed.

3.2.3. Floral morphology

The description of floral features of both colour variants was made after examining fresh flowers on the first day of flower opening. The observations on the following biometric characters were also recorded.

1. Length and breadth of sepals
2. Angle at the tip of sepal
3. Length and breadth of petals
4. Angle at the tip of petal
5. Length of filaments and anthers
6. Diameter of the stigmatic cup

The breadth was taken at the broadest part of the sepal and petal.

3.3. DETERMINATION OF ANTHER DEHISCENCE

The colour and appearance of anthers were observed with hand lens at hourly intervals from 6 am on the previous day of flower opening onwards in ten

fully mature flower buds until the dehiscence of pollen grains in each type, to find out the time of anther dehiscence (Prasad and Krishnaprasad, 1994).

3.4. DETERMINATION OF STIGMA RECEPTIVITY

The stigmatic surface was also observed for any change in colour or appearance in the same buds used for the above study at hourly intervals to find out the onset of stigma receptivity. Duration of stigma receptivity was also estimated as per standard procedures (Radford *et al.,* 1974). The presence of exudate in the stigmatic cup or its moist condition was considered as indication of stigma receptivity.

3.5. POLLINATION BIOLOGY

Three sets of flowers from each colour variant were used for this study. One set consisting of 10 fully mature buds were protected until the completion of anthesis. The buds were protected by covering them with butter paper cover and tying at the base two days before opening. Another set was emasculated but kept unprotected. The third set was taken as control. The extent of fruit set was recorded in all the sets under evaluation. Various insects visiting the flowers were also observed.

3.6. PALYNOLOGY

Pollen grains were acetolysed as per the procedures suggested by Nair (1970). The acetolysed pollen grains were microscopically examined to describe the shape, presence of aperture, exine sculpturing and any other special features. Pollen size was measured using phase contrast microscope.

Fertility of pollen was assessed on the basis of staining with acetocarmin-glycerin mixture (Moore and Webb, 1972). The pollen grains which were well stained were classified as fertile and others as sterile. Observations were taken from ten different fields for each type using microscope. The values were expressed as percentage

3.7. FRUIT DEVELOPMENT

The fruit development was studied after the submergence of flower in water in both the types after flowering.

Results and Discussion

4. RESULTS AND DISCUSSION

The results of the study 'Reproductive biology of water lily (*Nymphaea nouchali* Burm. f.)' carried out in Department of Plant Breeding & Genetics, College of Horticulture, Vellanikkara during 2010-2012 using two flower colour variants, blue and white, are presented below.

4.1. LEAF CHARACTERS

The leaves of both the colour variants of *Nymphaea nouchali* were simple, orbicular with subpeltate lamina, and deeply cleft near to the petiole base. The leaves were found to be glabrous on both the surfaces with wavy margin and 14 to 15 primary veins prominently raised beneath. The tip of leaf was obtuse or blunt. The petiole was long, slender and submerged in water with lamina floating on the water surface. The length of the petiole varied depending on the depth of water. Dassanayake (1996) also reported similar morphology for *Nymphaea nouchali* leaves.

In blue types the adaxial surface of the leaf was pale green with purplish irregular patches and abaxial surface green with dark purple spots and pale purple margin (Plate 1). However, the leaves of white types were green above and pale green beneath with purple colouration mainly towards the margin (Plate 2). The petiole was glabrous and brownish green in colour in both the types.

The observations recorded on various biometric characters like the length of petiole, length of lamina, length of notch, width of lamina at the base, middle and tip at the full expansion stage in the two colour variants of *Nymphaea nouchali* are presented in Table 1.

From the Table 1, it can be seen there was significant difference between the two colour variants for mean length of leaf as well as mean width of leaf at the middle and tip. The blue type was significantly superior to the white type in all the above mentioned characters with mean values 21.97±0.79 cm, 20.76±0.84 cm

Plate 1. Adaxial and abaxial surfaces of leaf in blue type

Plate 2. Adaxial and abaxial surfaces of leaf in white type

Table 1. Leaf characters of the two colour variants of *N.nouchali*

Type	Mean petiole length (cm)	Mean length of lamina (cm)	Mean length of notch (cm)	Mean width of lamina (cm)		
				At the base	Middle	At the tip
Blue	61.25±4.97	21.97±0.79	8.84±0.38	13.81±0.24	20.76±0.84	14.24±0.60
White	56.45±2.44	19.71±0.50	8.07±0.27	12.90±0.79	17.68±0.55	11.87±0.49
t-value	NS	2.43*	NS	NS	3.04**	3.05**

* Significant at 5% level** Significant at 1% level NS - Non significant

and 14.24±0.60 cm respectively. There was no significant difference among the two types in mean length of the notch, mean width of the lamina at the base and mean petiole length.

4.2. FLOWERING BIOLOGY

4.2.1. Growth Pattern of Flower Bud

The growth pattern of the flower buds in the two colour variants of *Nymphaea nouchali* represented by the mean number of days taken to reach water surface, the mean number of days to flower opening from their visual appearance on the surface of mud, the mean length of pedicel at the time of flower opening and after shedding, mean length and circumference of the flower bud at maturity, diameter of fully opened flower and blossom life are presented in Table 2.

The flower buds were found to be developing from the axils of leaves. There was no significant difference between the two types in the number of days taken by the flower bud to reach the water surface and to flower opening. In both types, it took almost six days for the flower bud to reach the water surface. Different stages in the development of bud in both the colour variants are shown in Plates 3 and 4. The flower opening occurred nearly three days after the bud reaching the water surface (Table 2). The fully mature flower buds in both the types are shown in Plates 5 and 6.

There was no significant difference between the types in mean pedicel length. It can also be seen from the Table 2 that even after flower opening the pedicel elongation continued in both the types to an extent of nearly 4 cm. Minimol (2004) also made a similar observation in *Nelumbo nucifera* Gaertn.

The rate of growth of pedicel in the case of two colour variants is presented in Fig.1. It can be seen that both the types followed the same pattern with the maximum growth rate on the day just prior to flower opening. However, the increment in growth of pedicel declined after flower opening.

Table 2. Growth pattern of flower buds in two colour variants of *Nymphaea nouchali*

Type	Days taken to reach water surface	Days to flower opening	Mean Pedicel length		Size of mature bud		Diameter of flower (cm)	Blossom life (Days)
			At flower opening (cm)	After shedding (cm)	Length (cm)	Circumference (cm)		
Blue	5.9±0.38	8.8±0.42	24.55±1.28	28.02±1.35	4.43±0.11	6.43±0.36	8.41±0.34	3
White	5.4±0.37	8.1±0.41	26.54±1.81	30.47±1.91	5.46±0.25	5.25±0.36	10.19±0.57	4
t-value	NS	NS	NS	NS	3.84**	7.31**	2.66**	#

** Significant at 1% level NS - Non significant # Statistitical analysis not done since all the values are equal

Plate 3. Different stages of bud development in blue type

Plate 4. Different stages of bud development in white type

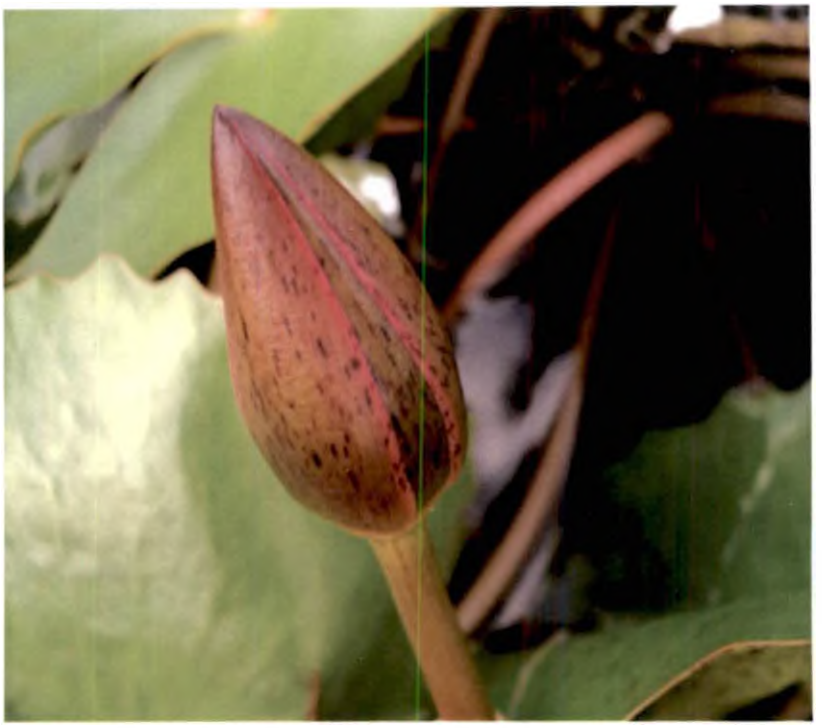
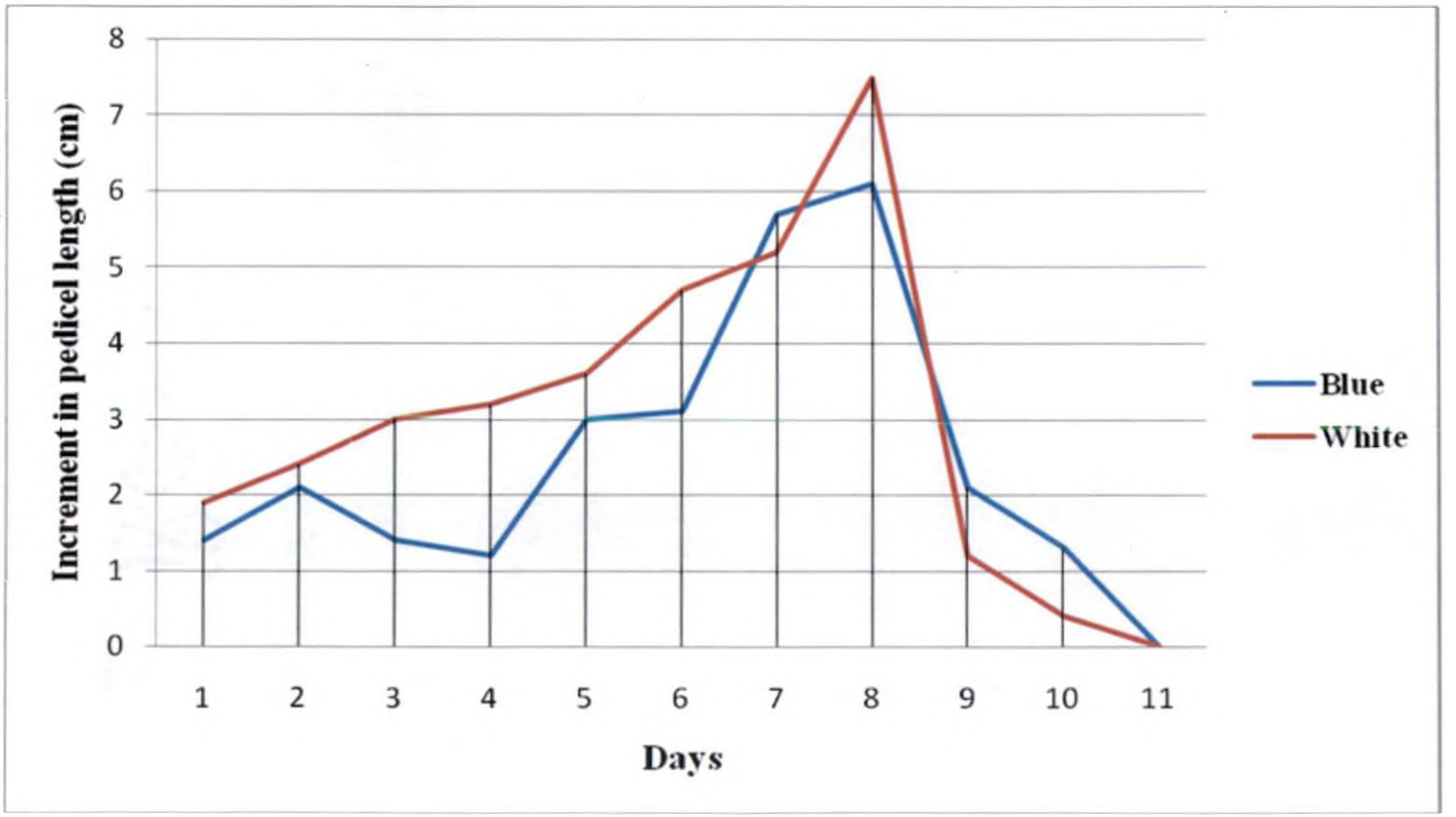


Plate 5. Fully mature bud of blue type

Plate 6. Fully mature bud of white type

Fig. 1. Increment in pedicel length in two colour variants of *Nymphaea nouchali*



The types differed significantly in the size of fully mature flower buds as well as opened flowers (Table 2). The white type produced larger flowers when compared to blue type. The flower buds of white types represented a mean length of 5.46±0.25 cm as against blue types with 4.43±0.11 cm. However, in the circumference of flower bud, blue type was superior with a mean value of 6.43±0.36 cm. The white types were found to be having larger flowers with a mean diameter of 10.19±0.57 cm. This can be attributed to the superiority of white type to the blue type in length of flower bud. Hence, it can be concluded that the diameter of the flower is proportional to the length of flower bud.

The flowers of both the types were found to be faintly fragrant. The blossom life was observed to be three days in blue type where as it was four days in white type.

The anthesis in both the types lasted for a few days. The flowers of both the types opened in the morning and closed in evening hours and again opened on the next day. The blue type opened consecutively for three days and on the fourth day it opened only partially and bent downwards. On the fifth day it sank completely into water along with the peduncle. The white type flowers opened and closed consecutively for four days and sank into water on fifth day. The successive stages of flower opening in blue type are presented in Plate 7 and white type in Plate 8. The floral parts did not dehisce but decayed fully into dark mucilaginous mass after 6-8 days (Plate 9).

The process of blooming began with the opening of the sepals. Depending up on the weather conditions the opening time of flower varied from 7.30 am to 9.45 am and it took 15-20 minutes for full blooming. The closing time varied from 5.15 pm to 6.15 pm and it took 10-15 minutes for full closing. Bright and sunny days favoured early opening of the flower in both the types. Begum *et al.*, (2010) also reported that opening and closing of flowers are very much influenced by sunlight and temperature. According to Prance and Anderson (1976),

Plate 7. Successive stages of flower opening in blue type

Plate 7a. First day of flowering

Plate 7b. Second day of flowering

Plate 7c. Third day of flowering

Plate 7d. Last day of flowering

Plate 8. Successive stages of flower opening in white type

Plate 8a. First day of flowering

Plate 8b. Second day of flowering

Plate 8c. Third day of flowering

Plate 8d. Last day of flowering

Plate 9. Decayed flower

temperature was more effective than sunlight in the opening and closing of *Nymphaea* flowers.

4.2.2. Periodicity of Flowering

The number of flowers produced by each colour variant per plant in each season expressed as percentage of total number of flowers produced per year is given in Table 3.

Table 3. Seasonal effect on flower production of two colour variants in *Nymphaea nouchali*

Type	Proportion of flowers produced (%)			
	Winter (Dec-Feb)	Summer (March-May)	Rainy (June-Aug)	Spring (Sep-Nov)
Blue	22.22	19.44	25.00	33.33
White	22.38	19.40	25.37	32.84

It can be seen that flowers were produced throughout the year in both the colour variants of *Nymphaea nouchali* under study. However, the flower production was found to be more (33.33% in blue type and 32.84% in white type) during September to November representing the spring season. Flower production was comparatively less in summer representing the months March to May. The weather parameters during the period of study are presented in Appendix III. Begum *et al.* (2010) reported that flowering in *Nymphaea nouchali* and *N. pubescence* mainly takes place from May to November, where as flowering in *Nymphaea rubra* occurs round the year.

The succession of flower production in each colour variant during the peak period is represented in Table 4.

Flowers were produced on an average of 3-4 days interval in both the types. No significant difference was observed between the types in the periodicity of flower production.

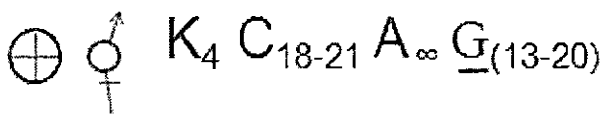
Table 4. Periodicity of flower production in two colour variants of *N.nouchali*

Type	Periodicity (days)
Blue	3.6±0.24
White	3.2±0.37
t-value	NS

NS – Non significant

4.2.3. Floral Morphology

The flowers were found to be solitary, pedicellate, actinomorphic, bisexual and complete with the floral formula



The comparison of morphological features of flowers of the two colour variants in *Nymphaea nouchali* is presented in Tables 5 to 8.

Four sepals, acute and in imbricate aestivation were present in both the colour variants. In blue type, sepals were dark green with purple streaks along the entire length on the outer surface. Inner side of sepal was found to be light purple in colour without any streaks. The base of the sepal on the inner side was light greenish colour (Plate 10). However, in white type the sepals were light green in colour on the outer surface and creamy white on the inner side (Plate 11).

The mean length of sepal varied significantly between the two types and was found to be higher (5.98±0.29cm) for white type (Table 5). No significant difference was observed between the types for mean breadth of the sepal as well as angle at the tip of the sepal.

Plate 10. Outer and inner sides of sepals in blue type

Plate 11. Outer and inner sides of sepals in white type

Table 5. Sepal characters in two colour variants of *Nymphaea nouchali*

Type	Mean number/flower	Mean length (cm)	Mean breadth(cm)	Angle at the tip
Blue	4	4.04±0.09	1.77±0.04	66.66±2.39
White	4	5.98±0.29	1.82±0.11	61.38±2.86
t-value	#	8.13**	NS	NS

** Significant at 1% level NS – Non significant # Statisticai analysis not done since all the values are equal

The petal characters of the two colour variants are presented in Table 6. The petals were seen to be boat shaped and arranged in a spiral fashion on the floral axis.

In blue type, petals were dark sky blue in colour. The petals of the outer whorl were characterized by the presence of dark brown streaks along the length (Plate 12). The base of petals in all the whorls of blue type were cream coloured.

Petals were bluish white in white type. The intensity of bluish tinge was more towards the tip of petal. The bluish tinge was maximum on the day of flower opening and reduced day by day and became pure white on last day of blooming. The petals of the outer whorl had a light green colour at the base which extended almost up to the middle of the petal. Small dark violet patches were seen on the outer surface of petals of the outer whorl (Plate 13).

The mean number of petals per flower was 19.50±0.27 for blue type and 19.80±0.80 for white type where as it was reported as 16.80±0.6 by Begum *et al.* (2010). The types did not differ significantly in the number of petals per flower.

In both the types the petals showed a gradation in size both in the length and breadth of units with the outermost whorl having the largest and the innermost whorl having the smallest petals (Plate 12 and 13). The types differed significantly in the length of the petals in different whorls. The white type was having larger petals in all the whorls when compared to the blue type. The length of the petal ranged from 5.62±0.32 cm in the outer whorl to 4.73±0.24 cm in the inner whorl in white type. The corresponding values in the blue type were

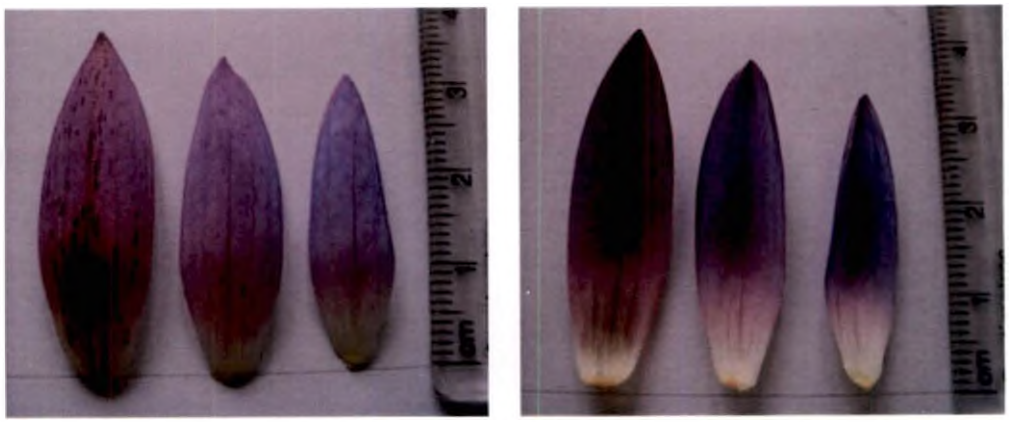


Plate 12. Outer and inner sides of petals in blue type showing gradation in size

Plate 13. Outer and inner sides of petals in white type showing gradation in size

Table 6. Petal characters in two colour variants of *N. nouchali*

Type	Colour	Mean number/flower	Mean length (cm)			Mean breadth (cm)			Angle at the tip (°)		
			Outer whorl	Middle Whorl	Inner Whorl	Outer whorl	Middle whorl	Inner whorl	Outer whorl	Middle whorl	Inner whorl
Blue	Blue	19.50±0.27	3.78±0.07	3.48±0.09	3.18±0.09	1.22±0.03	1.18±0.04	0.96±0.03	84.47±2.13	80.43±2.46	64.10±1.33
White	White	19.80±0.80	5.62±0.32	5.28±0.30	4.73±0.24	1.28±0.05	1.18±0.04	0.93±0.04	60.96±0.84	55.96±0.67	46.06±1.74
t-value		NS	7.56**	7.37**	7.43**	NS	NS	NS	7.54**	6.85**	7.99**

** Significant at 1% levelNS – Non significant

Table 7. Characters of androecium in two colour variants of *N. nouchali*

Type	Mean No. of stamen/ flower	Mean length of filament (mm)					Mean length of anther (mm)					Mean length of appendage (mm)				
		W_1	W_2	W_3	W_4	W_5	W_1	W_2	W_3	W_4	W_5	W_1	W_2	W_3	W_4	W_5
Blue	100.10±5.70	7.62	6.36	5.55	4.52	3.54	9.28	9.14	8.10	7.16	4.88	4.48	2.66	1.75	1.12	0.70
White	98.40±10.45	8.04	6.68	5.62	4.66	2.80	13.52	12.72	10.11	8.82	5.22	3.38	2.26	2.18	1.00	0

W_1- First whorl W_2- Second whorl W_3- Third Whorl W_4- Fourth Whorl W_5- Fifth Whorl

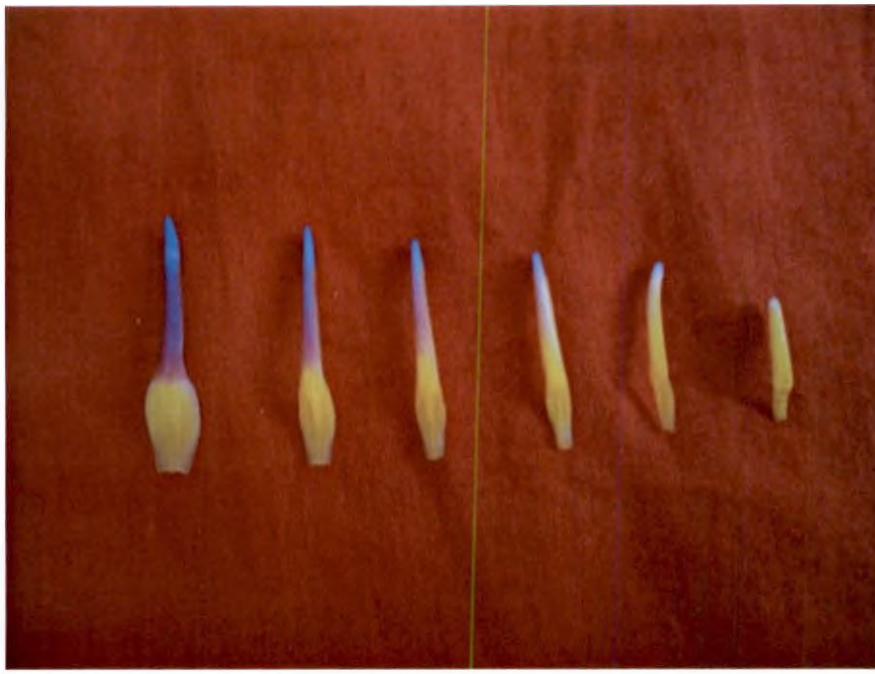


Plate 14. Stamens in blue type showing gradation in size

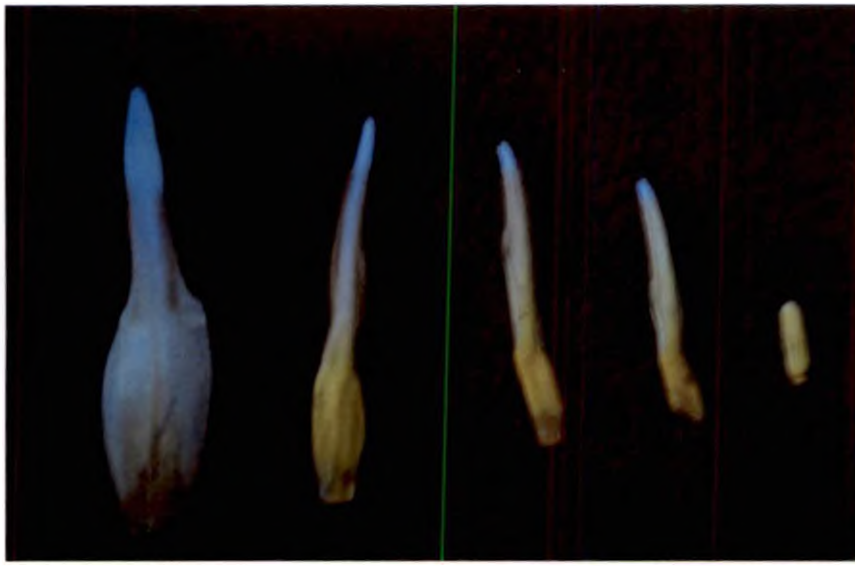


Plate 15. Stamens in white type showing gradation in size

Plate 16 . Dried up stamens in white type on the last day of opening

3.78±0.07 cm and 3.18±0.09 cm. There was no significant difference between the types in mean breadth of the petals.

Angle at the tip of petal was found to decrease as we proceed from the outer whorl to the inner whorl in both the types. Angle at the tip differed significantly among the types, with the white type having a higher angle of 84.47±2.13°, 80.43±2.46°, and 64.10±1.33° respectively in outer, middle and inner whorls (Table 6).

The staminal characters in two colour variants of *Nymphaea nouchali* are presented in Table 7. Numerous stamens arranged in a spiral fashion were present in both the types. Each stamen consisted of a filament, anther and a sterile appendage at the tip. The length of the stamen was the highest in the outer whorl and was found to decrease gradually towards inner whorl (Plates 14 and 15) in both the types. Unlike in blue type, stamens of the innermost whorl were devoid of appendages and the stamens of the outermost whorl were found to be dried up and dark in colour on the last day of flower opening (Plate 16) in white type.

The filaments of stamens in the outer whorl were found to be flattened with slight dilation at the base, dark yellow in colour and having a mean length of 7.62 mm and 8.04 mm for blue and white type respectively. It, however, decreased to 3.54 mm and 2.8 mm respectively in the innermost whorl (Fig. 2 and Table 7). The filaments of the outer whorl of stamens can be considered as slightly petaloid in nature (Plates 14 and 15).

The anthers were observed to be dark yellow and having a length of 9.28 mm in blue type and 13.52 mm in white type. There was a reduction in the length of anthers to 4.88 mm and 5.22 mm respectively in blue and white type (Fig. 3) as we proceed to the center of the flower. Purple colouration was observed on the inner side of stamens in blue type which was found to be absent in white type.

A light blue sterile appendage was seen at the tip of each stamen. It ranged in length from 4.48 mm in blue to 3.38 mm in white type in the outer whorl. The

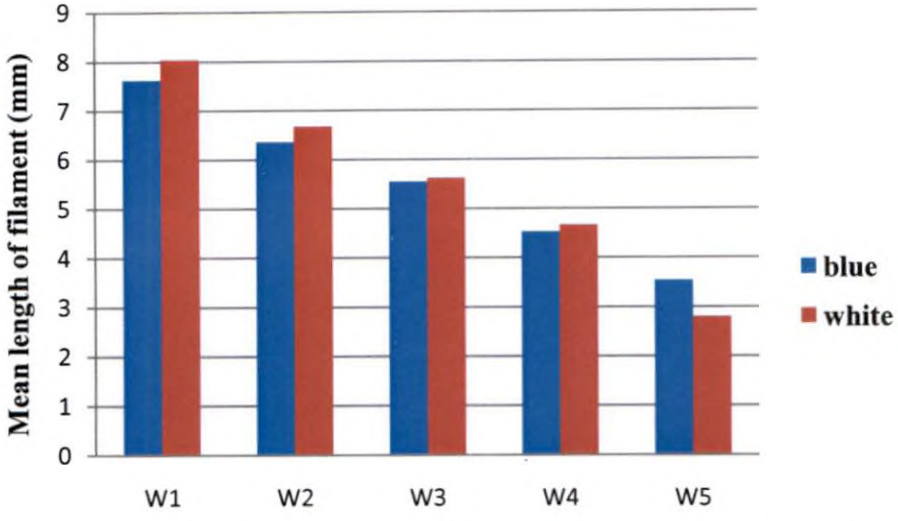


Fig. 2. Mean length of filament in two colour variants of *Nymphaea nouchali*

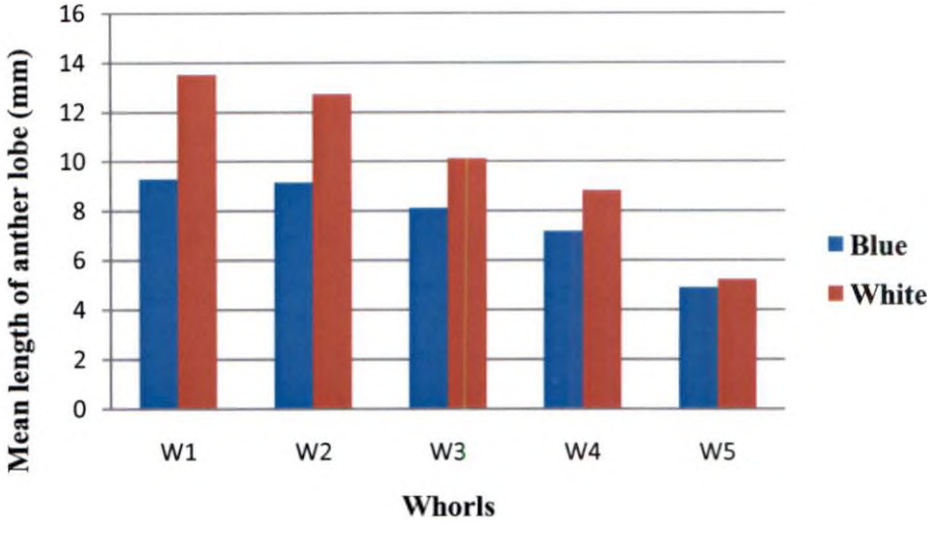


Fig. 3. Mean length of anther in two colour variants of *Nymphaea nouchali*

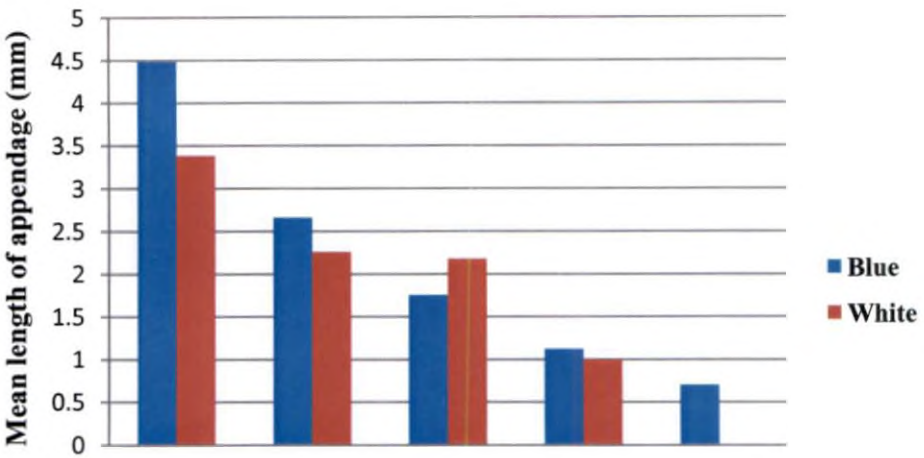


Fig. 4. Mean length of appendage in two colour variants of *Nymphaea nouchali*

size of the appendage was also found to decrease towards the inner whorls (Fig. 4). The colour of the appendage in white type was observed to be slightly lighter than that of the blue type (Plate 14 and 15).

The characters of gynoecium in the two colour variants under study are presented in Table 8.

In both the colour variants, gynoecium was found to be syncarpous (Plate 17) with yellow cup shaped stigma. A small white knob shaped receptacular tissue was found at the center of the stigmatic cup. Clavate appendages slightly curved inwards were present along the rim of the stigmatic cup (Plate 18). The number of the stigmatic appendages was found to be equal to the number of carpels in both the types. The diameter of the stigmatic cup varied significantly between the two types with 2.02±0.05 cm for blue type and 1.82±0.04 cm for white type. Begum *et al* (2010) reported that the diameter of stigmatic cup is 2.10±0.12 cm in blue type. Significant variability was also observed between the two types in the number of carpels which corresponded to the number of stigmatic appendages. It was 19.2±0.86 for white type and 15.2±0.39 for blue type. The number of carpels observed by Begum *et al.* (2010) in *N. nouchali* from Bangladesh was 13.55±0.45.

Table 8. Characters of gynoecium in two colour variants of *Nymphaea nouchali*

Type	Mean diameter of stigmatic cup (cm)	No. of carpels/receptacle
Blue	2.02±0.05	15.20±0.39
White	1.82±0.04	19.20±0.86
t-value	3.24*	4.94**

* Significant at 5% level ** Significant at 1% level

Plate 17. Cross section of ovary showing carpels

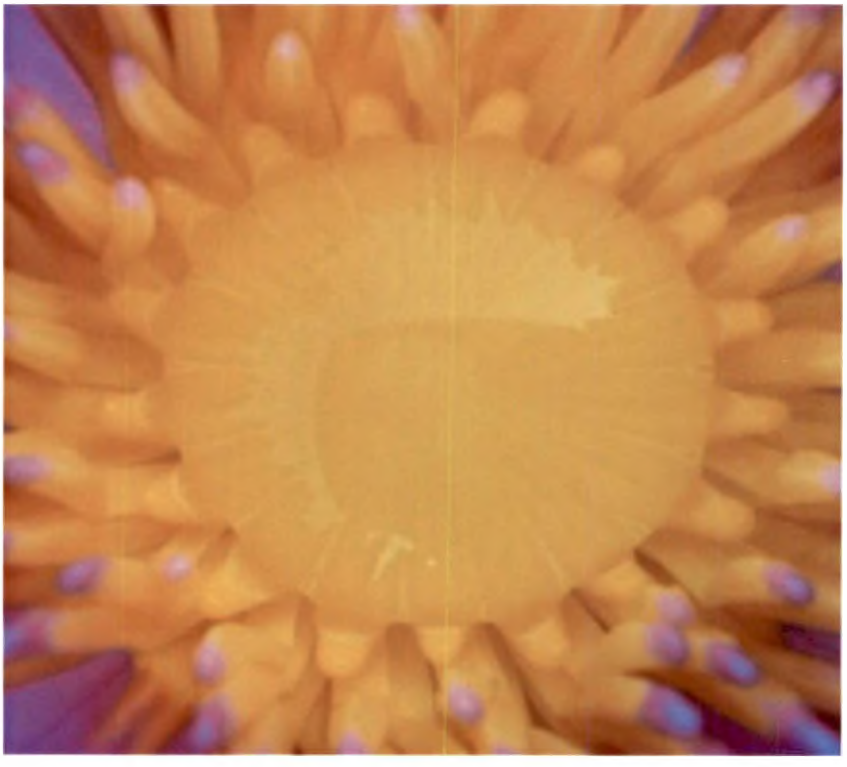


Plate 18. Stigmatic cup showing appendages

4.4. DETERMINATION OF STIGMA RECEPTIVITY

The stigmatic surface was examined for the presence of exudates at hourly interval starting from 6am on the previous day of flower opening. The honey dew like secretion found filling the stigmatic cup indicated the initiation of receptivity of the stigma (Plates 20 and 21). The exudates started to appear on the stigmatic surface between 3.05 pm and 4.10 pm on the day prior to flower opening. Stigma showing receptivity before dehiscence of anthers indicated the protogynous nature of flower. The protogynous nature of the Nymphaea flowers was also reported by Schneider (1982), Wiersema (1988). Begum *et al.*(2010) also observed protogyny in *N. rubra* and *N.pubescence*.

Stigmatic secretion was retained in the cup up to late evening (up to 7.45 pm to 8.00 pm) on the day of flower opening. The receptivity started almost 17 hours before opening of the flower and retained up to 32 hours even after flower opening. The stigmatic appendages remained reflexed out on the first day of flower opening fully exposing the stigmatic cup. On the second day of flower opening even though the exudates were absent, the surface of stigmatic cup remained moist indicating that receptivity is not lost. The loss of receptivity was indicated by the drying up and slight darkening of stigmatic cup along with inward curving of stigmatic appendages (Plate 22). This started by 3.40 pm to 4.30 pm on the second day of flower opening. The stigmatic appendages are thus found to play a significant role in the breeding behaviour of the species.

In the flowers of all the species observed by Begum *et al.* (2010) also showed the stigmatic cup was full of stigmatic exudates on the first day of anthesis. Prance and Anderson (1976) observed presence of the fluid on stigmatic cup. Meeuse and Schneider (1980) reported that its composition is optimal for pollen germination. The presence and role of exudates were also observed by Mosely (1961), Heslop-Harrison and Shivanna (1977) and Schneider (1982).

Plate 19. Anther dehiscence in the middle whorls of blue type

Plate 20. Receptive stigma in blue type

Plate 21. Receptive stigma in white type

Plate 22. Dried stigma with curved stigmatic appendages indicating the loss of receptivity

4.5. POLLINATION BIOLOGY

There was no seed set in any of the protected, unprotected or emasculated but unprotected buds.

Honey bees, house flies and weevils were found to be the insects visiting the flowers (Plates 23 and 24). The number of pollinators was found maximum on the second day of flowering. Among the honey bees which were the major insects visiting the flower, *Trigona irridipennis* was the predominant one. Tetali *et al* (2008) observed that the insects visiting *N. nouchali* were confined to Diptera and Hymenoptera. Capperino and Schneider (1985) also reported honey bees, flies and beetles as pollinators in *N. capensis* .

Several dead insects were repeatedly observed in the stigmatic cup of both the types of *N. nouchali* indicating the insectivorous nature of the flower. Due to the presence of exudates filling the stigmatic cup insects were trapped on the first day of flower opening. The exudates as well as the slippery and incurved nature of the stigmatic appendages prevented the escape of insects trapped in the stigmatic cup. The inner whorls of stamens with their unique structure and shape also acted as insect retaining mechanisms. The wet wings and body parts made swimming, flying or climbing up the anthers more difficult and the insects finally drowned. Several insects were seen to be trapped in the stigmatic cup at a time (Plate 25 and Plate 26).

In the subsequent days of flower opening no insects was trapped as the stigmatic cup dried up. The dead insects on the stigmatic cup were found to be dried up on the last day of flower opening (Plate 27). Insect cadavers were seen to be embedded and retained in the floral parts even one week after the submergence of the flowers (Plate 28). This type of insectivorous behaviour in *N. nouchali* was also described by Tetali *et al.* (2008). He also reported that this process was unique and found neither in other *Nymphaea* species nor in other species of plants. This behaviour according to him makes *N. nouchali* as the missing link in the evolutionary history of highly evolved carnivorous families.

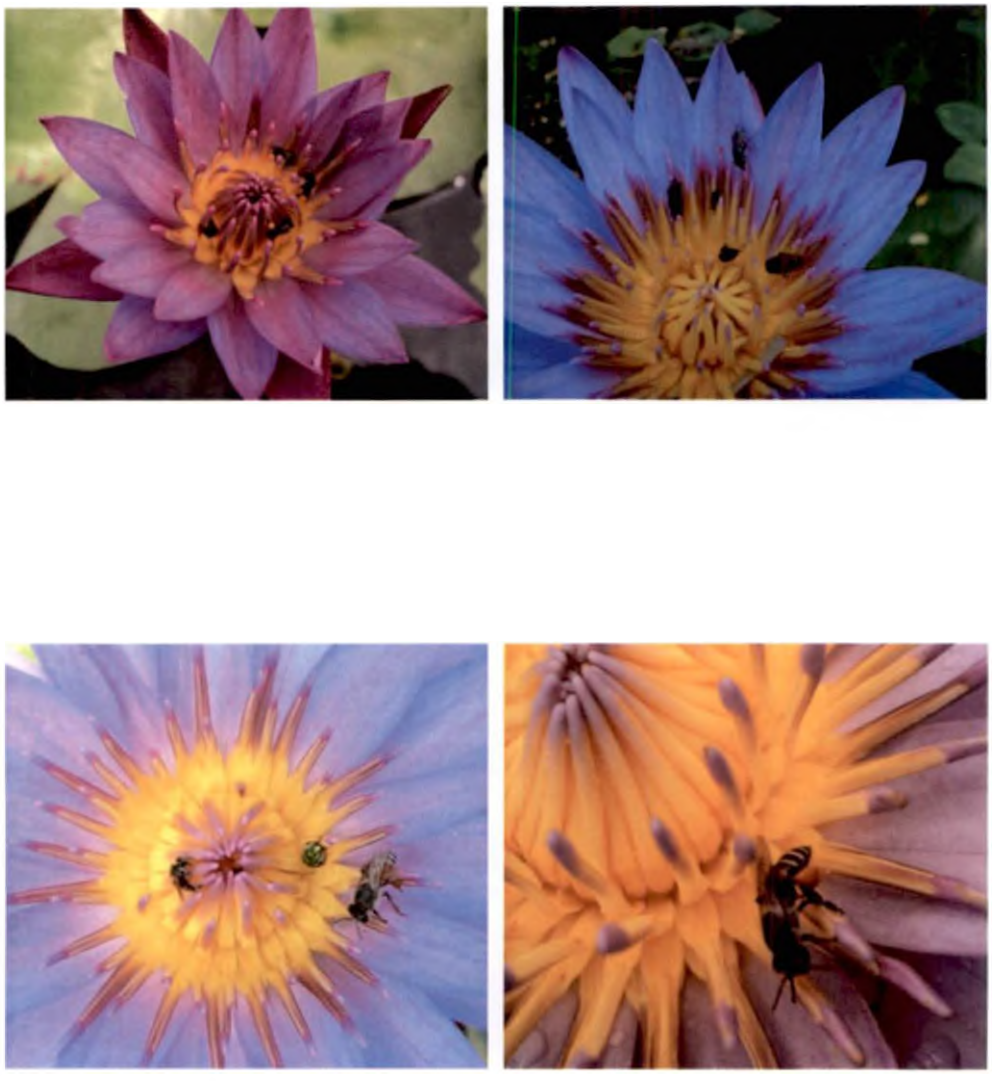


Plate 23. Insects visiting the blue type flowers

Plate 24. Insects visiting the white type flowers

Plate 25. Insects trapped in stigmatic fluid in blue type

Plate 26. Insects trapped in stigmatic fluid in white type

Plate 27. Dried insects in the stigmatic cup on the last day of flowering

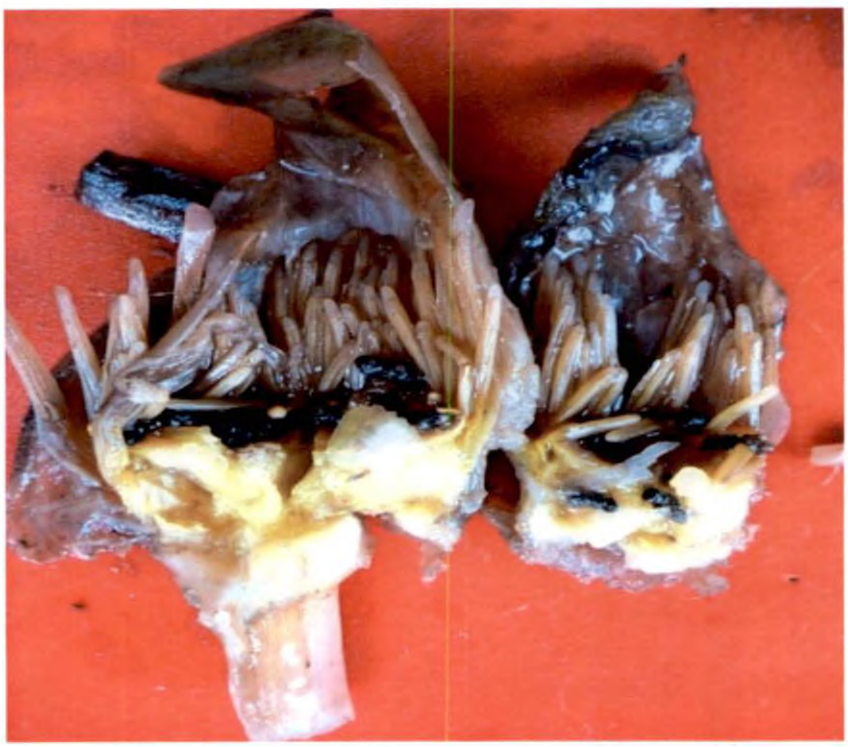


Plate 28. Insect cadavers seen inside the floral parts after submergence

4.6. PALYNOLOGY

The morphology, size and fertility of pollen grains in two colour variants are presented in Table 9 and Table 10.

The pollen grains were found to be round, yellow in colour, and monocolpate with reticulate exine in both the colour variants (Plates 29 and 30). There was no significant difference between the two types in the size of the pollen grains. Ansari *et al.* (2005) also reported monocolpate pollen grains in *N. nouchali.*

Table 9. Characters of pollen in two colour variants of *Nymphaea nouchali*

Type	Pollen type	Colour as appeared to naked eye	Size of fertile pollen	
			Length (µm)	Breadth (µm)
Blue	monocolpate	yellow	39.88±2.62	38.46±2.67
White	monocolpate	yellow	34.51±1.66	32.14±1.41
t-value			NS	NS

NS- Non significant

Table 10. Pollen fertility in two colour variants of *Nymphaea nouchali*

Type	First day of flower opening (Outer whorl) in %	Second day of flower opening (Middle whorls) in %	Third day of flower opening (Inner whorls) in %
Blue	3.45	5.53	3.33
White	5.86	8.27	6.99

Very low fertility was observed in both the types used for the study (Table 10). Pollen fertility was observed maximum in middle whorls, 2nd and 3rd whorls of stamen, on the second day of flower opening. The fertility was more in white type when compared to blue type. The germinability of pollen grains was hence, tried in 1M sucrose solution as well as in the stigmatic exudate. No germination

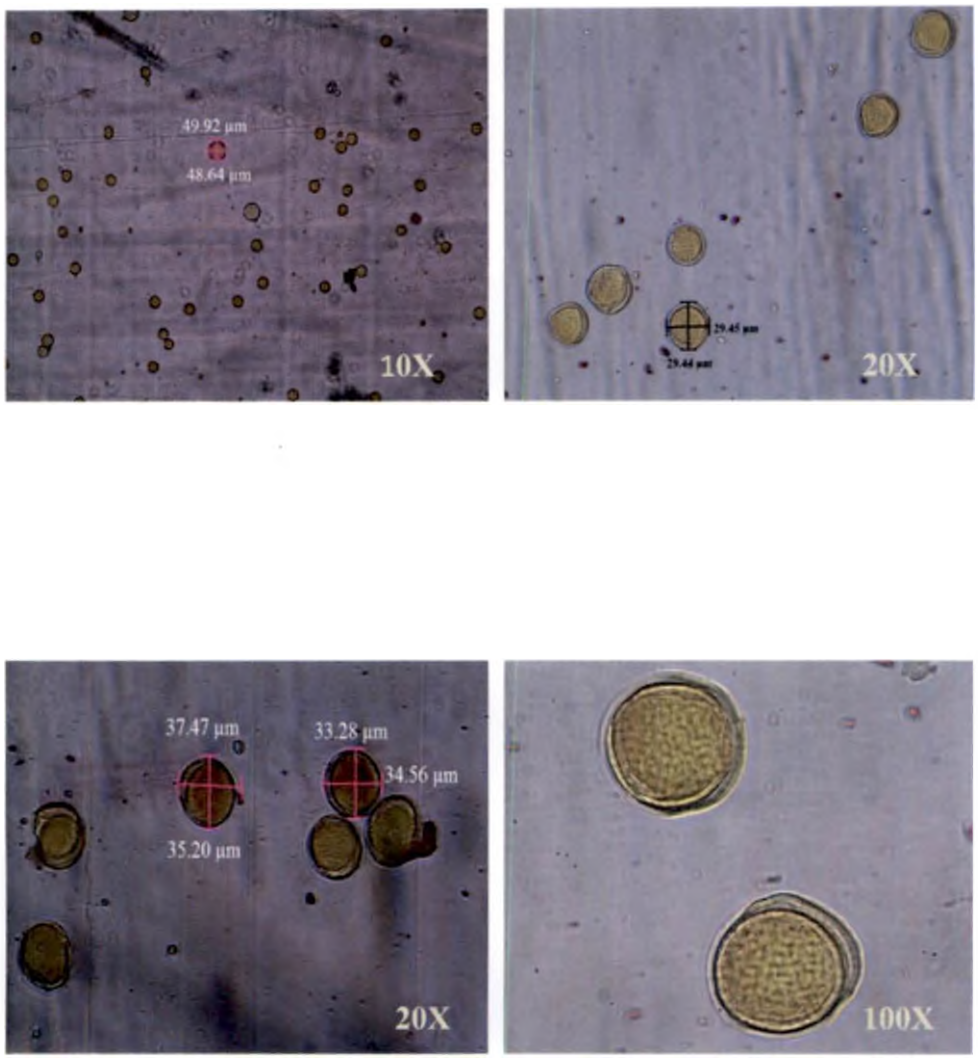


Plate 29. Pollen grains in blue colour variant of *Nymphaea nouchali*

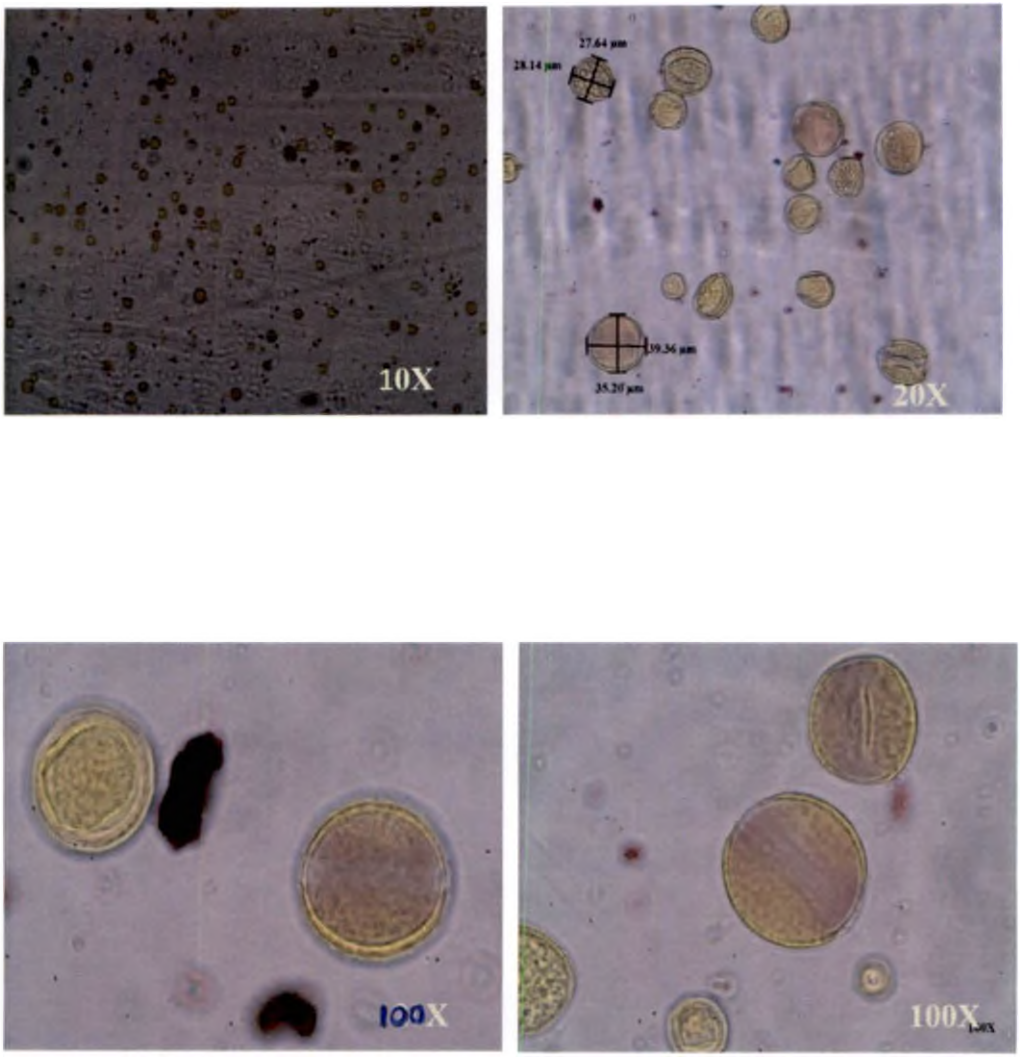


Plate 30. Pollen grains in white colour variant of *Nymphaea nouchali*

was obtained in any of the two media tried indicating the poor fertility of pollen grains. Ansari *etal.* (2005) has however, reported high pollen fertility in *Nymphaea nouchali*. Three types of *Nymphaea nouchali* namely, Type I, Type II and Type III with different types and levels of ploidy has been reported by Hossain *et al.* in 2007. This may be contributing to pollen sterility due to irregular meiosis. Considering this, further detailed cytological and palynological studies are to undertaken in *Nymphaea nouchali types* to unravel the cause of pollen sterility

4.7. FRUIT DEVELOPMENT

No fruit or seed development was observed in both the types. The flower fully decayed 6-8 days after sinking into water without fruit or seed formation (Plate 31). The very low fertility of the pollen grains may be inhibiting the fruit set in both the types.

Vegetative propagation through vivipary was found to be prominent in both the colour variants under study. The new plantlets are found to be arising from the upper portion of the mature leaf where petiole touched the lamina (Plate 32 and 33).The new plantlets remained attached to the petiole until the petiole decayed. There after they started growing independently by the elongation of roots. Even small flowers of 3.9-4.9 cm diameter and 11 to 14 petals could be found in one month old plantlets (Plate 34) in white types.

Plate 31. Decayed floral parts of *Nymphaea nouchali* without fruit or seed formation

Plate 32. Different stages of development of new plantlets from leaf in blue type

Plate 33. Different stages of development of new plantlets from leaf in white type

Plate 34. Flowers formed on a new plantlet from leaf

Summary

5. SUMMARY

The present investigation entitled 'Reproductive biology of water lily (*Nymphaea nouchali* Burm.f.)' was carried out in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara during the period 2010 to 2012.

Two different flower colour variants of *Nymphaea nouchali,* blue and white types, collected from Malabar Botanical Garden, Calicut, and maintained in cement tanks in the Department of Plant Breeding and Genetics were selected for the study. The salient findings of the study are summarized below:

- The leaves of both colour variants were observed to be simple, orbicular with sub-peltate lamina and deeply cleft near to the petiole base. The petiole was long, glabrous and brownish in colour with lamina floating on the water surface in both the types.
- There was significant difference between the two types in mean length of leaf as well as mean width of leaf at middle and tip. The blue type was superior to the white type in all the above mentioned characters.
- In both the types it took almost six days for the flower bud to reach the water surface and the flower opening occurred nearly three days after the bud reaching the water surface.
- There was no significant difference between the types in mean pedicel length. Even after the flower opening the pedicel elongation continued in both the types to an extent of 4 cm. Maximum growth rate of the pedicel was observed on the day just prior to the flower opening. The increment in pedicel elongation declined after flower opening.
- The types differed significantly in size of fully mature flower bud as well as opened flower. The white type produced longer flower buds and thus larger flowers when compared to blue type.

- The flowers of both types were faintly fragrant. The flowers opened in morning and closed in the evening hours and again opened on next day. The blossom life was three days for blue type where as it was four days in white type.
- The opening time of the flower varied from 7.30 am to 9.45 am. The closing time varied from 5.15 pm to 6.15 pm. Bright and sunny days favoured early opening of the flowers.
- The flowers were produced throughout the year. The flower production was more in spring season (September to November). The flowers were produced on an average of 3-4 days interval in both the types. Hence, this species can be well recommended for water gardens.
- Flowers were found to be solitary, pedicellate, and complete with various floral whorls in spiral fashion on the floral axis. There were four sepals, 18-21 petals, numerous stamens and 13 to 20 carpels for each flower.
- The length of the petals and stamens showed a gradation in size in both the colour variants under study with the outer most whorl having the largest and the inner most whorl having the smallest units.
- Each stamen consisted of a filament, anther and a sterile appendage at the tip. The initiation of dehiscence occurred by the longitudinal splitting of the anthers in both the types. The dehiscence of anthers proceeded from the outer whorl to the inner whorl of stamens. The time of dehiscence varied from 10.10 am to 11.30 am on the first day and the dehiscence was completed in 30 to 40 minutes.
- Stigmatic appendages curved inwards and equal in number to the number of carpels were present along the rim of the stigmatic cup. The appendages were more in white type while the stigmatic cup was bigger in blue type.

- The flowers were found to be protogynous. The stigma became receptive 17 hours before flower opening and the receptivity was retained up to 20 hours even after flower opening.
- Honey bees, house flies and weevils were found to be the major insects visiting the flowers.
- Several dead insects observed in the stigmatic cup of both the types indicated the insectivorous nature of the flower.
- The pollen grains were found to be round, yellow in colour and monocolpate with reticulate exine in both the colour variants. Very low fertility was observed in both the types.
- No fruit or seed development was observed in both colour variants. The absence of fruit or seed set can be attributed mainly to the very low fertility of pollen grains which needs further detailed investigations.
- The vegetative propagation from leaf was found to be prominent in both colour variants under study.

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APPENDIX I

Different vernacular names for *Nymphaea nouchali* in India

Languages	**Vernacular names**
Malayalam	Ambal Poovu
Tamil	Alli, Ambal, Allithamarai, Vellambal, Neelotpalam
Kannada	Neeltare
Telugu	Allithamara, Kaluvapoovu, Kaluva, Neelathamara
Hindi	Neel Kamal, Kumudinee
Sanskrit	Kumuda, Indivar, Nilakamala, Nilotpala, Utpala, Padma, kamala, Indeevararn
Urdu	Neelofer
Bengali	Kumud, Sundi
Panjabi	Neel Kamal, Kamalini
Gujarati	Poyanu
Marathi	Kamoda, Neel Kamal
Assamese	Bhoga bhet, Seluk

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Appendices

APPENDIX I

Different vernacular names for *Nymphaea nouchali* in India

Languages	**Vernacular names**
Malayalam	Ambal Poovu
Tamil	Alli, Ambal, Allithamarai, Vellambal, Neelotpalam
Kannada	Neeltare
Telugu	Allithamara, Kaluvapoovu, Kaluva, Neelathamara
Hindi	Neel Kamal, Kumudinee
Sanskrit	Kumuda, Indivar, Nilakamala, Nilotpala, Utpala, Padma, kamala, Indeevararn
Urdu	Neelofer
Bengali	Kumud, Sundi
Panjabi	Neel Kamal, Kamalini
Gujarati	Poyanu
Marathi	Kamoda, Neel Kamal
Assamese	Bhoga bhet, Seluk

APPENDIX II

Traditional uses of different parts of *Nymphaea nouchali*

Part	Traditional Uses
Whole Plant	Used for treatment of liver disorders in Ayurveda. Leaves, fruits and flowers are used for diabetes, blood disorders, antifertility, heart troubles, dysentery, eruptive fevers, indigestion and as a cardiotonic, emollient, diuretic, narcotic, stimulant and aphrodisiac. The flowers and roots have mild sedative properties and are used for mind-altering purposes. The whole plant is used as an anti-periodic and cardiac stimulant in Kashmir.
Flower	Flowers are used as in the treatment of diabetes mellitus and liver disorders in the Ayurveda and Siddha systems of medicine. The flower has bitter-sweet taste, removes impurities from the blood, cools and alleviates cough, used for vomiting, worm infestation and burning of the skin. The decoction of the flower is used against heart diseases and as narcotic. The syrup of the flower is used in cases of high fever and inflammatory diseases of the brain. The filaments of the plants are used as an astringent and a cooling agent for the burning sensation of the body.
Root	The roots are used as diuretic and to treat diabetes, infections of the urinary passages and infertility. Powder is used to treat diarrhea, dyspepsia and piles.
Leaf and peduncle	The tender leaves and flower peduncles are used in curries in Srilanka.
Rhizome and stem	Used for the treatment of diseases in urinary tract. It is often eaten in India and Srilanka. Rhizome paste is used to treat menstrual problems and gastrointestinal disturbances.
Seed	Restorative seeds are prescribed as a diet for diabetes mellitus in the Ayurvedic system of medicine.

APENDIX III

Weather parameters during the period of study

Month	Maximum Temperature (°C)	Minimum Temperature (°C)	Mean Relative Humidity (%)	Average Rainfall (mm)
June 2011	23.55	29.50	88.37	176.34
July	23.55	29.00	88.40	146.36
August	22.87	29.40	87.25	172.23
September	23.10	30.08	83.50	108.80
October	23.40	31.84	78.30	76.68
November	22.93	31.53	66.75	12.40
December	22.58	32.05	61.00	0.60
January 2012	21.50	32.50	56.40	0.00
February	22.18	35.33	56.75	0.00
March	31.88	35.20	67.25	0.88
April	24.82	34.38	73.40	20.38
May	25.30	32.60	76.00	117.30

REPRODUCTIVE BIOLOGY OF WATER LILY

(*Nymphaea nouchali* Burm.f.)

By

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ABSTRACT OF THE THESIS

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ABSTRACT

Water lilies are a group of fascinating aquatic perennial herb belonging to the genus *Nymphaea.* Among the different species of *Nymphaea, Nymphaea nouchali* is one common in south India. This species is commonly known as blue water lily, blue star water lily or star lotus. The plant is historically and functionally significant since it is associated with our culture and tradition. This species which forms an important constituent of aquatic flora possesses immense medicinal and ornamental values. Despite of its immense potentialities, water lily has received very little attention of crop improvement workers. Information on developmental pattern and reproductive biology which is fundamental for crop improvement programmes is lacking in this plant. Hence this investigation entitled 'Reproductive biology of water lily (*Nymphaea nouchali* Burm.f.) was under taken up with the objective of elucidating the reproductive biology and developmental pattern of flowers and fruits in *Nymphaea nouchali.*

Two flower colour variants of *Nymphaea nouchali*- blue and white types maintained in cement tanks were selected for the study. The study was conducted during 2010-2012 in the Department of Plant Breeding and genetics, College of Horticulture, Vellanikkara.

The leaves of both the colour variants are simple, orbicular with sub-peltate lamina and deeply cleft near to the petiole base. The petiole of both the type was long, glabrous and brownish in colour with lamina floating on the water surface. The blue type was significantly superior to the white type for mean length of leaf as well as means width of leaf at middle and tip.

In both the types it took almost six days for the flower bud to reach the water surface and the flower opening occurred nearly three days after the bud reaching the water surface. Even after the flower opening the pedicel elongation continued in both the types to an extent of 4 cm. Maximum growth rate of the pedicel was observed on

the day just prior to the flower opening. The white type produced longer flower bud and thus larger flowers when compared to blue type. However the circumference of the flower bud was more for blue type.

The flowers of both the types were faintly fragrant. The flowers opened in morning and closed in the evening hours and again opened on next day. The opening time of the flower varied from 7.30 am to 9.45 am. The closing time varied from 5.15 pm to 6.15 pm. Bright and sunny days favoured early opening of the flower. The blossom life was three days for blue type where as it was four days in white type. The flowers were produced throughout the year on an average of 3 to 4 days interval in both the types. Hence it can be well recommended for water gardens.

The flowers were found to be solitary, pedicellate, and complete with various floral whorls in spiral fashion on the floral axis. Significant variability was observed on various floral characters among the two types.

Each stamen consisted of a filament, anther and a sterile appendage at the tip. The initiation of dehiscence occurred by the longitudinal splitting of the anthers in both the types. The pollen dehiscence occurred only after complete opening of the flower. The stigma receptivity started 17 hours before flower opening and the receptivity was retained up to 20 hours after flower opening.

Honey bees, house flies and weevils were found to be the major insects visiting the flowers. Several dead insects were observed in the stigmatic cup of both the types indicated the insectivorous nature of the flower.

The pollen grains were found to be round, yellow in colour and monocolpate with reticulate exine in both the colour variants. No fruit or seed development were observed in both the colour variants. The absence of fruit or seed set can be attributed to very low fertility of the pollen and some incompatibility mechanisms in the flower. The vegetative propagation from leaf was found prominent in both the colour variants under study.