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STUDY ON REPRODUCTIVE CONSTRAINTS AND SEED CHARACTERISTICS OF *TERMINALIA PANICULATA* ROTH

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(Final Report of project KFRI RP 707/2015)

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PROJECT PARTICULARS

1.	Project No.	:	KFRI RP 707/2015
2.	Title	:	Study on reproductive constraints and seed characteristics of *Terminalia paniculata* Roth
3.	Special area of study	:	Identification of the constraints in formation of viable seeds and standardization of seed handling of *T. paniculata*
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CONTENTS

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Abstract

The study was undertaken to examine reproductive constraints and seed characteristics of *T. paniculata* with the objectives of identification of constraints in formation of viable seeds, and standardization of seed handling of the species. Mature trees were selected from Peechi-Vazhani Wildlife Sanctuary in Thrissur District, Kerala based on vegetative characteristics and reproductive capacity. Different phenophases were recorded regularly during 2015-18. Flower, fruit and seed production patterns were observed. Anthesis and stigma receptivity were studied under laboratory condition. Pollen viability and germination under different conditions were evaluated. Suitable breeding systems was identified. Maturity index of fruits indicating maximum viable seeds were determined. Seed handling techniques of the species was standardized.

Mature leaves were found throughout the year and flushing commenced along with the South-West Monsoon and completed with the North-East Monsoon. Fruiting initiated in December and ripening completed in April. Reproductive phenophase was for nine months. Flowering completed within six months (September-February) and fruiting in five months (December-April) prior to South-West Monsoon.

Blooming of flowers completed in 6 hours and anthesis in 19 hours. Opening of calyx in night (01.30 to 02.30 hr), erection of first 2 stamens during 02.30 to 03.30 hr, other 6 stamens erected during 05.30 to 06.30 hr, and last 2 stamens during 07.30 to 08.30 hr. Anthesis of first 2 stamens was during 09.30 to 10.30 hr, followed by 3 stamens during 16.30 to 17.30 hr. Anthesis of the last 5 stamens was during 03.30 to 04.30 hr of the second day. Pollen production was 16540±685.9 per flower with 16540:1 pollen-ovule ratio. Nine floral visitors were identified and most of them were active during morning (7.00 am to 11.00 am) and evening (3.00 pm to 5.00 pm) and few of them were found throughout the day (7.00 am to 5.00 pm) and the moths were active during night. Pollen germinability was higher in 30 per cent sucrose solution (90% germination), followed by 35 per cent (76% germination) and 25 per cent (65% germination).

Maximum pollen viability was noticed in pollens collected during 10.00 hr (80%) and it reduced reduced to 32 per cent (22.00 hr).

Xenogamy (cross pollination using pollens from another tree) was the efficient breeding system, which resulted in 77.78 per cent fruit setting. Less fruit setting (53.33%) was noticed in geitonogamy (cross pollination using pollens from the same tree). Negligible fruit setting was noticed in natural pollination (2.22%) and autogamy/self-pollination (1.11%). High fruiting rate in xenogamy and geitonogamy confirmed cross pollination is successful in *T. paniculata* and which naturally occur only with the help of sufficient number of pollinating agents.

Twenty eight 28 per cent of the flower buds attained the ripen fruit stage. However, only 1.32 per cent of the flower buds became viable fruits. The study showed that the futility of flower buds was very high (about 99%). About 106080±47039 fruits were produced in a tree. Average seed output was 1.32% of the total fruit production (1400). Mean germination rate was 1.4 per cent; hence, the mean reproductive capacity of a tree was 19.6.

The study confirmed that 16th week after seed setting when the fruits become red is the best seed collection period to get viable seeds (2.6%). Seed emptiness was very high (96-98%) in the species. Pretreatment is not required for seed germination of the species. However, de-winging and water soaking helped slight improvement in seed germination. Although, seeds can be stored up to 6 months. It is advisable to sow them within one month after collection for getting better germination.

Overall regeneration of the species was 24.15 individuals per ha. Generally, about 52 per cent of the regeneration was under unestablished (seedlings) category (<3 cm girth class), 30% belonging to the established (saplings) category (3-9.9 cm girth class) and 18% in advanced (poles) category (10-30 cm girth class). It is indicated that overall regeneration of *T. paniculata* in the study sites was good (seedlings > saplings > poles). However, the regeneration is not at par with mother trees. The study concluded that cross pollination between trees and harvesting of fruits at 16th week after fruit setting (red-coloured fruits), *i.e.*, the optimum maturity period are the suitable conditions to obtain more viable seeds, which favours regeneration and thus helps to conserve the species.

1. INTRODUCTION

Terminalia is the second largest genus of the family Combretaceae comprising about 100 species distributed all over the tropical regions of the world. The term "*Terminalia"* originated from the Latin word "*Terminus*" referring that the leaves appear at the tips of the shoots. This tree genus is known for considerable economic importance in terms of wood and non-wood produces like tannins and are used in traditional folk medicine (Thangaraja & Ganesan, 2012). Most of them were used in traditional medicine (Fahmy *et al*., 2015).

About 24 species of *Terminalia* have been reported from India (Srivastav, 2003). Of them six species are naturally found in different forest types of Kerala *viz*., *T. bellerica* (Gaertn.) Roxb. (Semi-evergreen and moist deciduous forests), *T. chebula* Retz. (Dry and moist deciduous forests), *T. cuneata* Roth - *Syn*.: *T. arjuna* Roxb. ex DC. (Dry deciduous forests), *T. elliptica* Willd. - *Syn*.: *T. crenulata* Roth (Moist and dry deciduous forests), *T. paniculata* Roth (Moist and dry deciduous forests) and *T. travancorensis* Wight & Arn. (Evergreen forests). Other two species *viz*., *T. catappa* L. and *T. procera* Roxb are grown as ornamental trees. Among the species, *T. paniculata* is low regenerative one with poor regeneration status (Swarupanandan & Sasidharan, 1992; Chandrashekara *et al*., 1998; Pillai & Chandrashekara, 2011).

Terminalia paniculata is one of the large sized tree species. It is native to Peninsular Indian sub-continent and is commonly distributed in semi-evergreen and moist deciduous forests in Southern Indian States such as Karnataka and Kerala at the elevation up to 1200 m (Roth, 1821; Wight & Arnot, 1834; Brandis, 1874; Clarke, 1878; Cooke, 1903; Talbot, 1911; Chandrabose, 1983; Gangopadhayay & Chakrabarty, 1997; Sasidharan, 2004; Pillai *et al*., 2012; Abhilash *et al*., 2013; Nanda *et al*., 2014). It grows in area within the range 22-30^{0}C, though it can tolerate 19-39^{0}C. The species is commonly known as Kindal in terms of timber and Flowering Murdah in terms of ornamental purposes. Fruit is samara having 3 unequal

wings (middle one is larger). Ripened fruits become deep red with single brown seed (Sasidharan, 2004; Tilney & Wyk, 2004; Thangaraja & Ganesan, 2008 & 2012). The deep red coloured fruit gives awesome colouration to the entire forest area. The species is recently neotypified by the Botanical Survey of India (Chakrabarty & Kumar, 2017).

Terminalia paniculata is one among the important species used in timber industry. Wood is very hard and used for construction, furniture, cabinet, ship building, agricultural implements, mine props, fuel wood, etc. (Gamble, 1928; Mohanan & Sharma, 1985; Tilney & Wyk, 2004; Thangaraja & Ganesan, 2012). Bark and fruits are used for dyeing and tanning. Chemical composition and constituents of the species analysed on the basis of medicinal importance (Quattrocchi, 2012). Phytochemical studies on bark, leaf and fruits of *T. paniculata* revealed the presence of phenolic compounds - alkaloids, coumarins, terpenoids and high content of anti-oxidants. Anti-microbial study revealed that it is susceptible to Klebsiella pneumoniae, Bacillus cereus, Escherichia coli, Pseudomonas *aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, etc. (Raju & Rajasekhar, 2016). Bark has anti-oxidant, anti-inflammatory, anti-adipogenic, anti-diabetic, anti-HIV, anti-lipase, anti-obesity and hepato-protective activities (Eesha *et al*., 2011; Agarwal *et al*., 2011; Ramachandran *et al*., 2013; Sahil *et al*., 2013; Savithramma, 2013; Talwar *et al*., 2013; Goudar *et al*., 2014; Mopuri & Meriga, 2014; Mopuri *et al*., 2014; Mopuri *et al*., 2015; Durge *et al*., 2016; Narayan & Rai, 2016; Rajasekhar & Raju, 2016; Ganjay *et al*., 2017). Various phyto-chemical studies reveal usefulness of the species in maintaining wellness of the body.

Allozyme diversity of *T. paniculata* in different populations in Tamil Nadu was documented using biotechnological tools (Thangaraja & Ganesan, 2007). Earlier studies reported that natural population of the species is limited (Pillai *et al*., 2012; Thangaraja & Ganesan, 2012, Pillai, 2017). Compared to other species in the genus, *T. paniculata* shows very poor regeneration and low germination both in natural as well as laboratory conditions. Seed emptiness is common among the

genus and is very high in *T. paniculata*, where only 3% of the seeds are viable (Chacko *et al*., 2002). Pollination study by Thangaraja and Ganesan (2012) documented the morphology, pollen-ovule relationship, viability and gene flow among different populations of *T. paniculata.*

Population of *T. paniculata* in the native range is declining due to overexploitation and poor regeneration status. The endemic tree species, *T. paniculata* having lot of beneficial characteristics requires urgent attention and support to maintain the natural population in the native range. However, detailed studies to understand reproductive constraints of the species are scanty. In this context, the study was undertaken to examine reproductive constraints and seed characteristics of *T. paniculata* with the following objectives:

Objectives:

1. To identify constraints in formation of viable seeds in *Terminalia paniculata*
2. To standardize seed handling techniques of the species

2. MATERIALS METHODS

2.1. Selection of mother trees

In order to study the reproductive biology and seed characteristics of *T. paniculata*, an elite population of the species is required with sufficient number of individuals. Hence, a reconnaissance survey was conducted in the Peechi-Vazhani Wildlife Sanctuary, (Thrissur District, Kerala) and identified populations of the species. Mature mother trees were selected from the populations based on vegetative characteristics (straight, cylindrical, non-twisting bole, non-forking, free from buttresses, flutes, diseases, damages, etc.) and reproductive capacity (Kokutse *et al.*, 2016). All the trees with above features were selected and labelled for further data collection.

2.2. Phenology

Vegetative and reproductive phenophases were recorded regularly during 2015-18 on the selected mother trees of *T. paniculata* in Peechi-Vazhani WLS area. Reproductive phenophases include flowering and fruiting phases. Recorded data on leafing, bud formation, development and maturation of flower and fruits. Identified four branches of the selected trees in North-East-West-South direction, marked and labelled for counting the reproductive stages at regular intervals and recorded phenological events once in a week. Also determined flower, fruit and seed production pattern to assess reproductive capacity of the species.

2.3. Floral Biology

Reproductive phenophases were recorded visually in the field conditions. Thirty flower buds in each direction for all the four directions (North, East, West and South) of the selected mother trees were marked and tagged at the time of bud initiation as done in earlier investigations (Raju & Ramana, 2009; Raju *et al.*, 2011;

Raju *et al.*, 2012). Regular observations were made on the identified floral buds and recorded data on opening pattern of flower, anthesis, stigma receptivity, pollination, agents of pollination, fruiting, etc. Floral visitors were collected and recorded. Assessment of fruit development stages and fruit production pattern was done. Anthesis and stigma receptivity were studied under laboratory condition. Hourly data were recorded for opening of corolla, anthesis and stigma receptivity.

2.4. Pollen-viability and germination

Inflorescence with mature flower buds were collected from the selected mother tress before anthesis and brought to laboratory. In order to determine the duration for maximum pollen viability, flower buds were collected from 10.00 am to 01.00 am in the next day with an interval of three hours. Pollens were extracted by nicking end portion of the flower buds and mixed well. Different concentrations of sucrose solution were prepared to evaluate the pattern of pollen germination in *in-vitro*. Pollens were kept in different concentrations of sucrose solutions (05 to 50% with 5 per cent interval). Three microscopic slides were prepared for each concentration and three microscopic fields were observed per slide. Pollen germination was determined by taking mean of all the nine readings (Raju *et al.*, 2014).

2.5. Breeding mechanism

In order to identify the breeding system, different pollination experiments were conducted. Ten mother trees of the species were selected from Peechi-Vazhani Wildlife Sanctuary. Four inflorescences were selected in each tree and tagged. Separate experiments were conducted on each inflorescence. Inflorescences were bagged by thick water-proof paper bags before anthesis and artificially pollinated in three inflorescences. One inflorescence was subjected for natural pollination.

Monitored the fruit setting pattern in each inflorescence. Following experiments were carried out as per standard methods (Saunders & Sedonia, 2006):

1. Natural Pollination (N) - flowers were tagged and left to open pollination (Control)
2. Autogamy (A) - flower buds were bagged with minute holes and isolated from visitors (to test for self-pollination)
3. Xenogamy (X) - emasculated and artificially pollinated using pollens from another tree (to test for out-crossing ability)
4. Geitonogamy (G) - emasculated and artificially pollinated using pollens from the same tree (to test for self-compatibility).

2.6. Seed maturity index

Maturity index of fruits/seeds indicates the stage at which they attained a sufficient stage of development with maximum viable seeds. In order to determine the optimum maturity period, seeds of *T. paniculata* were collected at 25 different developmental stages (weekly interval) from the period of fruit setting and tested for viability in terms of cutting tests and germination tests. Seeds collected during each stage were subjected to germination trials under laboratory condition. The data compiled and determined the optimum stage for collection of most viable seeds.

2.7. Reproductive capacity

Reproductive capacity of a species is important as any other specific ecological features. It is defined as product of the average seed output and the fraction represented by the average percentage germination and is determined by the following formula (Salisbury, 1942).

$$\text{Reproductive capacity} = \frac{\text{Average seed output}}{100} \times \text{Average germination \%}$$

2.8. Seed Moisture Content

Seeds of *T. paniculata* were collected from the study area during ripened stage. High constant temperature oven-dry method was used to calculate the seed moisture continent (ISTA, 2014). The seeds were dried in hot-air oven for 1 hr at 130^{o}C. Calculated moisture content (MC %) on fresh weight basis as follows:

Moisture Content (MC %) = $(M_2-M_3) \times 100 / (M_2-M_1)$

where, M_1 = weight of the container with lid (g)

M_2 = weight of the container with lid and seed sample before drying (g)

M_3 = weight of the container with lid and seed sample after drying (g)

M_2-M_3= Moisture loss

M_2-M_1= Fresh weight of seed sample

2.9. Seed weight and size

Fresh seeds of *T. paniculata* collected from 10 mature trees and mixed thoroughly and weighed the seeds separately using electronic weighing machine. Seed weight was determined by weighing 8 replications of 100 seeds. Variance, standard deviation and coefficient of variation were calculated as follows (ISTA, 2018):

Variance= $n\{(\sum x^2)-(\sum x)^2\}/n(n-1)$

where, x = weight of each replicate in g

n = number of replications

$\sum$= sum of

Standard variation (S.D.) = $\sqrt{}$variance

Coefficient of variation (CV) = $(S.D. \times 100)/ \bar{x}$

where, $\bar{x}$ = mean weight of 100 seeds

If the CV value is less than four, weights of the 8 replication are valid.

2.10. Rapid viability and seed emptiness

Fruits of *T. paniculata* (1000 fruits in 8 replications) were subjected to cutting tests using seed cutter and observed visually. Rapid viability and seed emptiness was estimated by counting number of seeds present in each replication.

2.11. Germination

Ripened seeds were collected from mature trees and subjected to germination trails. Seeds were sown in vermiculite in plastic trays (n = 1000 seeds in 4 replications) and kept under laboratory condition. Different pre-sowing treatments were performed to study the effect of pre-treatment on seed germination.

Pre-sowing treatments were as follows:

Sl. No.	Treatment
1.	Seeds without any treatments (Control)
2.	Water-soaking for 12 hrs
3.	Water-soaking for 24 hrs
4.	Water-soaking for 48 hrs
5.	De-winged and water-soaking for 12 hrs
6.	De-winged and water-soaking for 24 hrs
7.	De-winged and water-soaking for 48 hrs

Temperature of water used for the treatment was approximately about 30^{o}C. Recorded data till culmination of germination and determined GID (Initial Day of Germination), GD (Germination Duration) and GP (Germination Percentage) as per standard methods (Coolbear, 1991; Bewley, 1997; Xu *et al.*, 2016). Germination related parameters were determined as follows:

GID = Dg - Ds (where, Dg = first germination day; Ds = seed sowing day)

GP = (G/T) x 100 (where, G = no. of germinated seeds; T = no. of seeds sown)

GD = Gf – Gi (where, Gf = final day of germination; Gi = initial day of germination)

2.12. Regeneration study

Regeneration pattern in natural condition was estimated by enumeration in three selected populations in Kerala state *viz.* Nedumkayam (Nilambur South Forest Division, Malappuram Dt.), Anamooli (Mannarkkad Forest Division, Palakkad Dt.) and Peechi (Peechi-Vazhani Wildlife Sanctuary, Thrissur Dt.). Sample plots (50 m x 50 m) were laid in Nedumkayam; Anamooli and Peechi forest areas for regeneration survey and the plots were established using the stratified random sampling method. Stratification of the sample plot is based on the appearance of tree density, coverage and occurrence of saplings. All the regeneration of the target species in the sample plots was enumerated.

2.13. Statistical analysis

Data on seed germination parameters such as germination percentage, treatment effect, seed maturation and regeneration were statistically analysed with the help of statistical tool, SPSS (16.0 version 22). One-way ANOVA is performed on the result of the study and critically analysed with the help of statistical tools, which helps to find out best seed collection period and need for pre-treatments to enhance seed germination.

3. RESULTS AND DISCUSSION

3.1. Population identification and mature tree selection

Large sized populations of *T. paniculata* were identified in the study area such as Jandamukku, Thamaravellachal, Seethal, Muniyara and KFRI Peechi Campus. The species is distributed as one of the important components in moist deciduous forests as well as semi evergreen forests (Pillai & Chandrasekhara, 2011; Pillai *et al.*, 2012, Pillai, 2017). Reconnaissance survey helped to identify populations of the species in the study area and selection of mature mother trees for the study. The study could generalize reproductive nature as well as the fruiting pattern in the species. The study helped to estimate reproductive capacity, seed production, optimum period for collection of viable seeds and identification of reproductive constraints.

3.2. Vegetative and reproductive phenophases

Figure 1 depicts vegetative as well as reproductive phenophases of the mother trees of *T. paniculata*. The figure represents various phenological events such as development of leaves, flowering and fruiting. Development of leaf was noticed during June-October. Flower buds initiated during September and development continued up to January. Fruiting initiated during December and ripening completed on April.

The study revealed that mature leaves of *T. paniculata* were found throughout the year. The flushing (leaf buds) commenced along with the South-West monsoon (June-September) and leaf maturation continued during the period and completed with the North-East monsoon (October-November). Flowering completed within six months (September-February) and fruiting in five months (December-April) before South-West monsoon. May is the dormant period, which make the tree ready for leaf bud initiation during monsoon and prevent the unfavourable external conditions such as high temperature, low water availability etc. (Kaur *et al.* 2013). The study showed that the vegetative

phenological events continued for six months (June to November); whereas, reproductive phenophase was for eight months (September to April).

Duration	L_B	L_Y	L_M	FL_B	FL_Y	FL_M	FR_B	FR_Y	FR_M
June	■	■	■						
July	■	■	■						
August		■	■						
September		■	■	■					
October		■	■	■	■				
November			■	■	■	■			
December			■	■	■	■	■		
January			■		■	■	■	■	
February			■			■	■	■	■
March			■				■	■	■
April			■					■	■
May			■						

Fig. 1. Phenophases in *T. paniculata*

***Note**: L_B= Leaf bud stage; L_Y = Young leaf stage; L_M = Mature leaf stage; FL_B = Flower bud stage; FL_Y = Young flower stage; FL_M = Mature flower stage; FR_B = Fruit bud stage; FR_Y = Young fruit stage; FR_M = Mature fruit stage)*

3.3. Fruit production

Period of seed production on parent plant can be one of the most hazardous phases in a plant's lifecycle. In many plants, only a very small proportion of the ovules eventually mature into viable seeds. This is because, many flowers fail to produce fruits, and many of the ovules in fruits fail to produce seeds. Studies on vide range of species have recorded huge variations in fruit/seed set (Wiens, 1984; Sutherland, 1986). Table 1 represents the quantification of various stages of flower and fruits production in *T. paniculata*. An average of 30±2 flowers recorded in a single unit of a panicle and 52224±3558 in a primary branch. Only 27.81% of the primordia of flowers developed into mature fruiting stage and 1.32% viable seeds. In a primary branch of inflorescence, 14523±989 ripened fruits and 689±4 seeds are formed. Result of the study exhibited futility of the flower buds at

different developmental stages as: about 31% at young flower stage, 44% at mature flower stage, 60% at fruit bud stage (fruit initiation stage), 64% at young fruit stage, 72% at mature fruit stage (ripened fruit) and 99% at seed stage (viable seeds).

The results showed that flower bud initiation started during second week of September and massive floral bud production started during the last week of September. These reproductive phases was more or less same in all the populations undertaken for the study. The fruit bud stage initiated during the fourth week of November and development ends during the second week of April. Flower and fruit production rate estimated that about 28% of the flower bud primordia attained the ripen fruit stage, which is very low compared to other tropical flowering trees (Singh & Kushwaha, 2006). However, only 1.32% of the flower bud primordia developed viable fruits. The study revealed that the futility of flower buds was very high, i.e., about 99 per cent. Various stages of flower-fruit production in *T. paniculata* are represented in Figure 2.

Table 1: Number and percentage (in parenthesis) of flower buds developed to fruit in a terminal unit of a panicle

Population	FL_B	FL_Y	FL_M	FR_B	FR_Y	FR_M	Seed
P1	34 (100%)	24 (70.59%)	20 (58.82%)	16 (47.06%)	14 (41.18%)	11 (32.35%)	0 (0%)
P2	30 (100%)	27 (90%)	21 (70%)	18 (60%)	16 (53.33%)	12 (40%)	2 (6.67%)
P3	28 (100%)	17 (60.71%)	14 (50%)	9 (32.14%)	7 (25%)	5 (17.85%)	0 (0%)
P4	31 (100%)	18 (58.07%)	16 (51.61%)	12 (38.70%)	10 (32.25%)	8 (25.80%)	0 (0%)
P5	28 (100%)	18 (64.29%)	14 (50%)	10 (35.71%)	8 (28.58%)	6 (21.42%)	0 (0%)
Mean	**30.2 (100%)**	**20.8 (68.89%)**	**17 (56.29%)**	**13 (43.04%)**	**11 (36.42%)**	**8.4 (27.81%)**	**0.4 (1.32%)**

Note: *P= Population; FL_B = Flower bud primordial stage; FL_Y = Young flower stage; FL_M = Mature flower stage; FR_B = Fruit bud stage; FR_Y = Young fruit stage; FR_M = Mature fruit stage; S = Seed*

Flower Bud Primordia

Flower Buds

Mature Flowers

Young Fruits

Semi-mature Fruits

Ripen Fruits

Fig. 2: Different stages of flowering and fruiting

An average of five primary branches were recorded in a tree with 3 secondary, 8 tertiary and 8 quaternary branches in a primary branch. Quaternary branch ends with 6-20 terminal units. Hence, an average of 5760 to 19200 (12480±9503) terminal units was in a tree. Terminal unit had 5 to 12 (8.5±4.95) fruits and estimated fruit production in a tree as 28800 to 230400 (106080±47039). Reproductive capacity of a tree was calculated as: average seed output multiplied by average germination percentage.

Average seed output (1.32% of the average fruit production of the tree)

$$= (106080 \times 1.32) \div 100 = 1400$$

Average germination percentage = 1.4 %

$$\text{Reproductive capacity} = \frac{1400}{100} \times 1.4 = 19.6$$

3.4. Pollination Biology

3.4.1. Anthesis

Terminalia species are bisexual or hermaphroditic in nature with male and female reproductive structures on the same flower. Though the male and female structures are in a same flower, self-pollination could not produce viable embryos, *i.e.*, self-incompatibility. *T. paniculata* is a hermaphroditic protandrous type with male structure contains ten stamens arranged in two rows. Female reproductive structure encloses one celled ovary with 2-3 ovules. Blooming in *T. paniculata* occurred in three intervals during 01.30 hr to 08.30 hr; opening of calyx during 01.30 to 02.30 hr in night (1 hour duration); followed by erection of first 2 stamens in next one hour (02.30 to 03.30 hr), erecting other 6 stamens during 05.30 to 6.30 hr, followed by the last 2 stamens during 07.30 to 08.30 hr. The events showed that a minimum of 6 hours was required for complete erection of all the 10 stamens in a flower.

Anthesis was between dawns of first day to night of next day as 09.30-10.30 (Day 1), 16.30-17.30 (Day 1) and 03.30-04.30 (Day 2). Anthesis of first 2 stamens was during 09.30 to 10.30 hr, followed by 3 stamens during 16.30 to 17.30 hr *i.e.*, 15 hours after calyx opening. Anthesis of the last 5 stamens was during 03.30 to 04.30 hr of the second day. Estimation of pollen production resulted those 1300-1800 (1614±63.29) pollens per anther and 12500-18800 (16540±685.9) pollens per flower. Pollen-ovule ratio is 16540:1. Compared to other species like, *T. arjuna* (15400:1) and *T. chebula* (10890:1), the pollen-ovule ratio is higher in *T. paniculata* (David *et al.*, 2012; Sankanur *et al.*, 2014). The study found that blooming of flowers completed within a period of 6 hours and anthesis in 19 hours. The rate of pollen production was higher (16540±685.9 pollens/flower) than that of other entomophilous species. Pollen productivity in other entomophilous species is reported as: *Toona ciliate* - 6445, *Vitex negundo* - 3684, *Jacaranda mimosifolia* - 2508 and *Azadirachta indica* - 10320 (Khanduri & Sharma, 2001; Chitnis & Chitnis, 2015). Similarly, pollen-ovule ratio was also higher in *T. paniculata* than that of *T. arjuna* and *T. chebula* (David *et al.*, 2012; Sankanur *et al.*, 2014).

3.4.2. Floral visitors

Figure 3 and 4 represent floral visitors actively pollinating during various time regime. Nine floral visitors (pollinators) belonging to Hymenoptera (Order of Wasps, Bees & Ants), Diptera (Order of Flies) and Lepidoptera (Order of Butterflies & Moths) including night-active moths were identified. They normally visit the flowers for foraging nectar, pollen or both. Most of the floral visitors were active during morning (07.00 hr to 11.00 hr) and evening (15.00 hr to 17.00 hr) and few of them were found continuously from morning to evening (07.00 hr to 17.00 hr). Black ants (*Camponotus compressus*) were the major visitors during 07.00 hr to 17.00 hr followed by Red ants (*Oecophylla smaragdina*) during 08.00 hr to 16.00 hr. House flies were also noticed as visitors during 08.00 hr to 12.00 hr. Moths are active only during night time (20.00 hr to 04.00 hr). No other floral visitors were reported during 18.00 hr to 06.00 hr except moths. Though, many visitors were observed some of them may not be involved in pollination.

Common Name	Scientific Name	Time of floral visit (24 hr clock)										
		07	08	09	10	11	12	13	14	15	16	17
Indian bee	*Apis cerana*											
Rock bee	*Apis dorsata*											
Little bee	*Apis florea*											
Red ant	*Oecophylla smaragdina*											
Black ant	*Camponotus compressus*											
House fly	*Musca domestica*											
Wasp	*Vespula vulgaris*											
Carpenter bee	*Xylocopa* sp.											

Fig. 3. Period of floral visitors in 24 hr duration

Fig. 4. Floral visitors (Black ant, House fly, Moth and Wasp)

Floral visitor were very few in *T. paniculata* compared to other trees species such as *Combretum constrictum*, *T. elliptica* (*T. tomentosa*), *T. bellerica*, *T. chebula*, *T. arjuna*, *T. catappa*, *T. pallida* (Chauhan *et al.*, 2008; Raju *et al.*, 2012; Talwar & Bhatnagar, 2014; Ekeke & Agbagwa, 2015; Gargi & Sinha, 2017) which resulted an ineffective cross pollination (xenogamy and geitonogamy) in *T. paniculata*.

Insect pollinated (entomophilous) flowers are attractive to insects in many ways as: conspicuousness, scent, nectar, edible sap and edible pollen and sticky pollens with rough surface. The entire genera (*Terminalia*) including *T. paniculata* is characterized by attractive scented white flowers with large amount of nectar, edible sap and pollen. Even though the size of the flower is very small, the plant shows gigantic blooming and golden appearance with scent, which is very attractive to the insect pollinators.

3.4.3. Pollen germination

Figure 5 depicts the pattern of pollen germination in various concentrations of sucrose solution. The results showed that maximum germination recorded in sucrose solution with 30% concentration. Above 50% germinability was recorded in three sucrose conditions such as 25, 30 and 35 per cent concentration and the least germinability was recorded in sucrose solution with 50 per cent concentration.

The *in vitro* study on pollen germination revealed that germinability of pollen was higher in 30% sucrose solution, followed by 35% and 25%. Germinability of pollen increased up to 35% sucrose solution and thereafter decreased. Ninety per cent of the pollens were germinated in 30% concentration of sucrose and 76 per cent in 35% concentration of sucrose. A similar study on *Terminalia arjuna* as maximum pollen germinability reported in 12.5% sucrose solution (David *et al.* 2012).

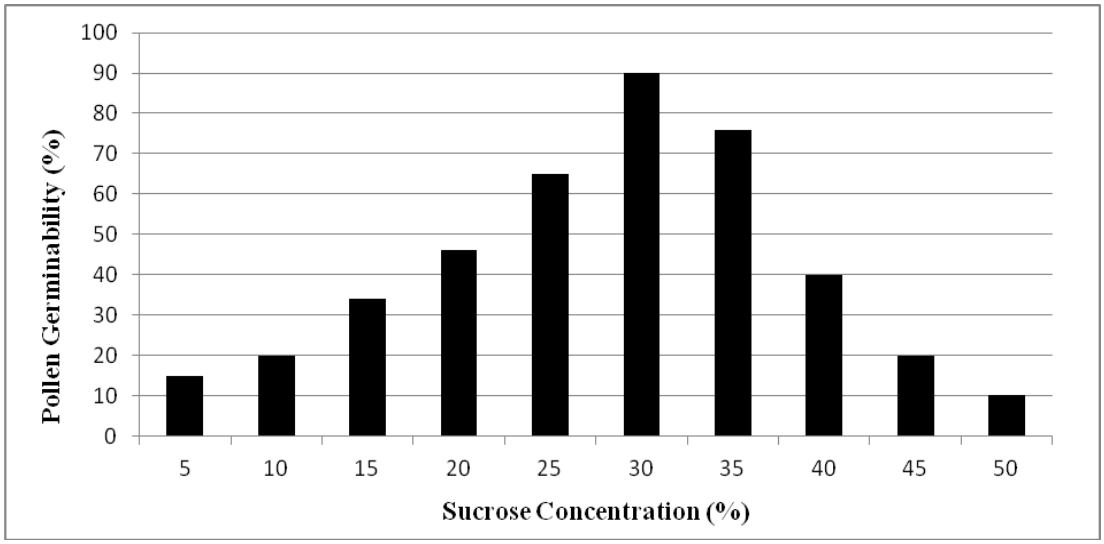

Fig. 5. Pattern of pollen germination in different concentrations of sucrose solution

3.4.4. Pollen viability

Pattern of pollen viability is depicted in Figure 6. The result showed that the maximum viability was in pollens collected during 10.00 AM. Viability of pollens gradually decreased from 80 to 30 per cent at 10 PM and completely lost thereafter. The study indicated that viability of pollens remains only for 12 hours.

The study revealed that pollen viability was higher in pollens collected during morning and non-viable at midnight collection. Maximum viability (80%) was in pollens collected during 10.00 hr. Viability reduced to 73% after 3 hours (13.00 hr), 61% after 6 hours (16.00 hr), 47% (19.00 hr) and 32% (22.00 hr). Pollen viability in other species reported as – Teak, 92.2% and gradually decreases from 11.00 hr to 3 days (84 hr), Almond species ranges from 66.7% to 94.5% in different populations, 71% in *Jatropha curcus* and later it is reported as 95% at 9 hr after anthesis and thereafter declined (Tangmitcharoen & Owens, 1997; Bayazit *et al*., 2011; Kaur *et al*., 2011; Abdelgadir *et al*., 2012). Some other studies reported pollen viability in different species such as Rubus (12-59%), Chestnut (67%), hazelnut (49-97%), apricot (76-86%), sweet cherry (67-81%), sour cherry (71%), walnut (81-94%) (Naybom, 1985; Seiidov 1988; Beyhan & Odabas, 1995; Bolat & Pirlak, 1999; Sutyemez, 2007).

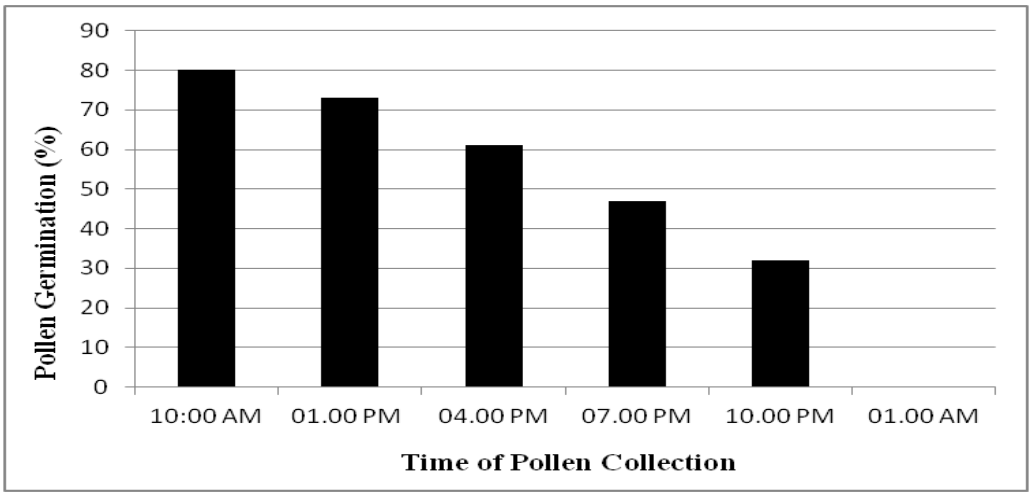

Fig. 6. Pattern of pollen viability in various time regime

3.4.5. Stigma receptivity

During opening of flower, the style elongated to about 1 mm in first day, which grown up to 3 mm in second day and 4 mm in third day. Flowers get dehydrated and fall down after the third day and became inactive. Fruit production rate was calculated in each day after flower opening resulted that no fruit formed during the first and second day of flower opening. Third/last day of flower opening, plant shows 80% of fruit formation.

3.5. Breeding Mechanisms

Results of different breeding methods such as natural pollination (N), Autogamy (A), xenogamy (X) and geitonogamy (G) depicted in Figure 7. The study revealed that xenogamy (cross pollination using pollens from another tree) is the efficient breeding mechanism, which resulted 77.78 per cent fruit setting. Less fruit setting (53.33%) was noticed in Geitonogamy (cross pollination using pollens from the same tree). Negligible fruit setting was noticed in natural pollination (2.22%) and Autogamy/self-pollination (1.11%).

Result of the study showed that xenogamy is the most successful breading mechanism in *T. paniculata*, followed by geitonogamy. Natural pollination and Autogamy is not fruitful in the species. The high fruiting percentage in xenogamy and geitonogamy confirmed that cross pollination is successful in *T. paniculata* and which naturally occur only with the help of sufficient number of pollinating

agents (Atluri & Rao, 2000; Atluri *et al*., 2003). Other studies also reported that low fruit/seed-set in plants may be due to either low pollinator density or low pollinator visitation rate (Schemske, 1980; Willson & Schemske, 1980; Howell & Roth, 1981).The reproductive constraints in *T. paniculata* might be due to the failure of natural pollination and breeding system. Hence, artificial pollination like xenogamy is recommended for maximum seed setting of the species.

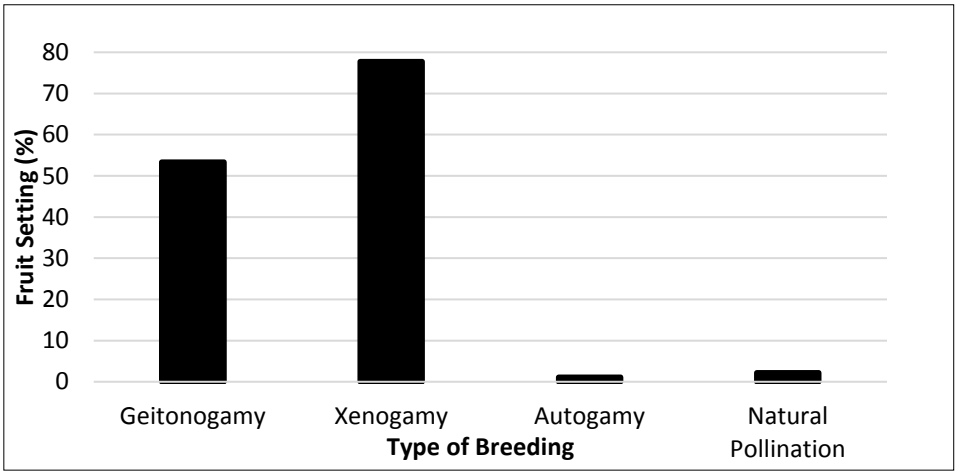

Fig. 7. Pattern of fruit setting in different breeding mechanisms

3.6. Seed Characteristics

3.6.1. Seed morphology

The fruit is a samara (an independent dry indehiscent fruit in which flattened wing of fibrous/papery tissue develop from the ovary wall) having three unequal wings with one larger and other two equally sized smaller ones. Average fruit size is 14.53 x 8.27 mm and the distance between two smaller wings 9.45 mm. This study showed that weight of a single seed of *T. paniculata* was 3.45 - 8.39 g and the number of seeds per kg was 11,909 - 28,908. Earlier report that seeds of the species is weighed about 26,103 – 59,966 seeds per kg (Gupta, 1937). Size of the fruit and seed is very small compared to other species of *Terminalia* such as *T. arjuna*, *T. bellerica*, *T. catappa*, *T. chebula*, *T. elliptica* and *T. travancoricus* (Chacko *et al*., 2002). Small size and low weight helps to seed dispersal through wind (Anemochory). Winged fruit morphology is also supports wind dispersal.

3.6.2. Maturity index

Maturity index is important for effective seed collection and it is varied with species. Figure 8 depicts germination pattern of seeds collected at one week interval from fruit setting. Table 2 represents the pattern of seed germination and moisture content under various maturity stages. The result showed that 16th week after fruit setting had maximum germination (2.6%) with 5.9 per cent moisture content and the fruit maturation period was between 11th and 25th weeks after fruit initiation. With reference to colour pattern of fruits, the maximum germinability was noticed in red coloured fruits with dark brown seed (at 16th week). The germination tests resulted that seed viability at 11th week after fruit initiation was 0.6 per cent and it increased 2.6 per cent at 16th week with 5.9 per cent moisture content. Thereafter, gradually decreased to zero per cent at 25th week with moisture content below 3.5%. Mean seed moisture content was 4.80±0.71% during the 11 - 24th weeks. The study confirmed that 16th week after seed setting is the best seed collection period for the most viable seeds, *i.e.*, the optimum maturity period. Fruits with red colour are the maturity index for collecting the most viable seeds. Earlier study by Pillai and Pandalai (2015) reported that maturity index is the change of fruit colour from green to yellow for *Artocarpus hirsutus*, attaining greenish-yellow fruit colour for *Calophyllum inophyllum* and bright yellow fruits with longitudinal furrows for *Dysoxylum malabaricum*.

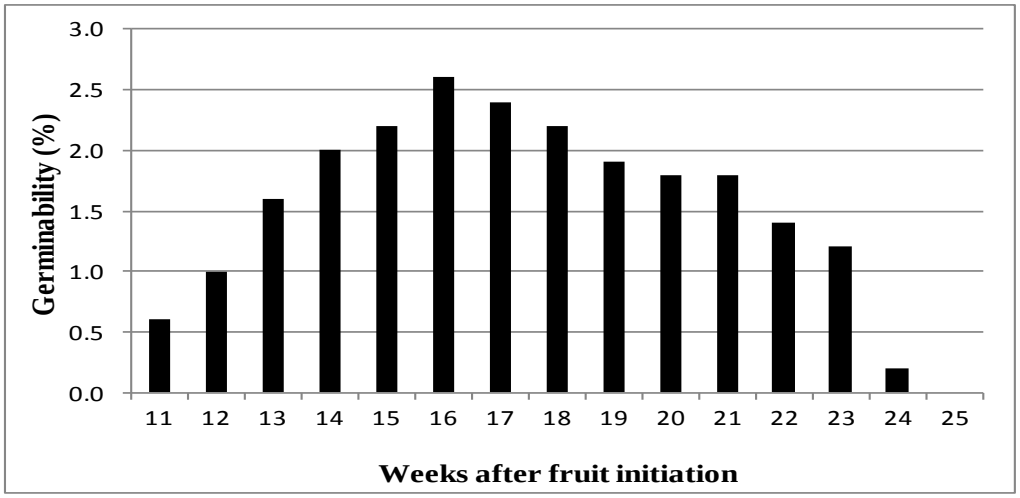

Fig. 8. Germination pattern of seeds under different stages of maturity

Table.2. Germination and moisture content of seeds under different stages of maturity

Fruit Colour	Week after fruit setting	MC%	Ger. %
Greenish red	11	4.4	0.6
	12	4.7	1.0
Light red	13	5.0	1.6
	14	5.3	2.0
Red	15	5.6	2.2
	16	5.9	2.6
	17	5.6	2.4
	18	5.3	2.2
	19	5.0	1.9
	20	4.7	1.8
Deep red	21	4.4	1.8
	22	4.1	1.4
	23	3.8	1.2
	24	3.5	0.2

3.6.3. Seed viability and emptiness

Rapid viability of seeds was estimated through manual seed cutting test, which showed 2-4 percentage of the fruits bearing seeds. The result indicated that seed emptiness was very high (98-96%) in the species, *T. paniculata* than any other members of the genus *Terminalia* as well as the family Combretaceae. This finding is substantiated with earlier study (Pillai & Chandrasekhara, 2011). The study resulted that the species showed very low viability rate and very high seed emptiness. Seed emptiness rate is very high compared with other species belongs to the taxa *Terminalia* and other associated tree species such as *Xylia xylocarpa*, *T. elliptica*, *Lagerstroemia microcarpa*, *Tectona grandis*, *Pterocarpus marsupium*, *T. bellerica*, *Grewia tilifolia* etc (Chacko *et al*., 2002; Pillai & Chandrasekhara, 2011).

The study showed 2.6 per cent germination in seeds of *T. paniculata* that collected during 16th week after fruit initiation. In earlier studies germination rate of the

species reported as <1 to 15 per cent (Gupta, 1937; Murali, 1997; Pillai & Chandrasekhara, 2011). According to Chacko *et al*. (2002), the poor viability might be due to seed dormancy and high seed emptiness. The present study showed that three weeks were required for completion of seed germination. Figure 9 depicts the trend of emerging germinant during days after sowing (DAS). Out of the total germinant (2.6%), 0.8 per cent of them was germinated during 14 DAS, followed by 0.6 per cent (15 DAS), 0.5 per cent (16 DAS) and 0.3 per cent (17 DAS), 0.2 per cent (18 DAS), 0.1 per cent (19 DAS and 0.1 per cent (20 DAS). The data confirmed that 85 per cent of the total germinant emerged within a period of 17 DAS.

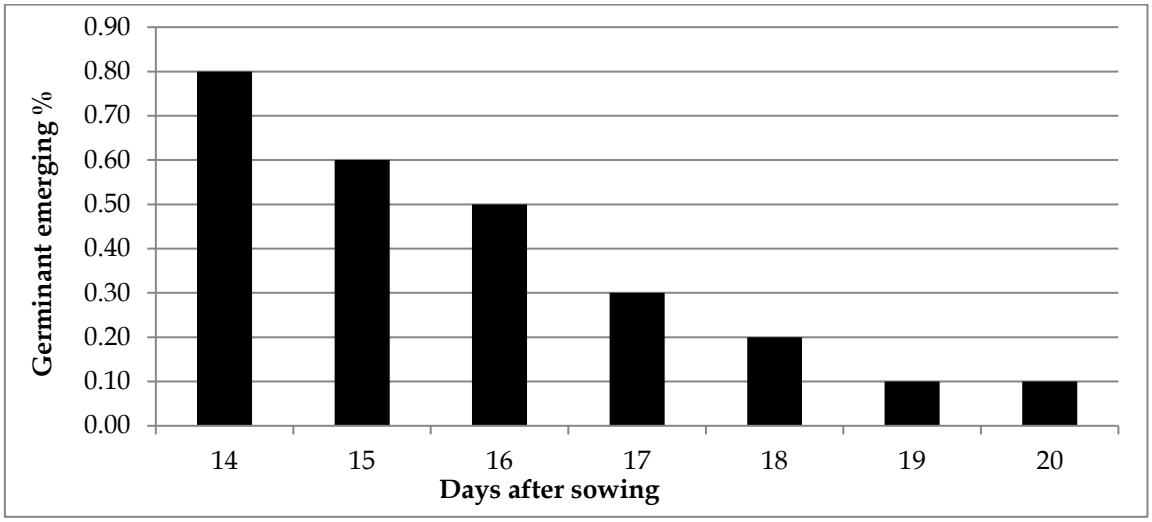

Fig. 9. Trend of emerging germinant during different days after sowing

3.6.4. Pretreatments

Result of the seed germination under different pre-treatments is presented in Table 3. The germination within these pre-treatments were analysed by using one-way ANOVA resulted that there is no significant difference between these treatments (*P≤0.13381*). The study revealed that there was no significant influence on pretreatment in seed germination of *T. paniculata*. Therefore, pretreatment is not required for seed germination in the species. However, de-winging and water soaking helped to slight improvement in seed germination of the species. Generally, water soaking is one of the pretreatments used for

enhancing seed germination in species like *T. bellerica*, *T. chebula* and *T. catappa,* and de-winging for *T. elliptica*, *T. arjuna*, etc. (Chacko *et al*., 2002). However, water soaking for overnight can be applied in *T. paniculata*.

Table 3. Germination pattern of fully ripened fruits of *T. paniculata*

Sl. No.	Treatment	No. of seeds sown	No. of germi-nants	Ger. %	Ger. period
1	Samara (Control)	1000	24	2.4	22
2	Samara soaked in water for 12 hr	1000	24	2.4	22
3	Samara soaked in water for 24 hr	1000	26	2.6	21
4	Samara soaked in water for 48 hr	1000	25	2.5	21
5	De-winged samara soaked in water for 12 hr	1000	26	2.6	21
6	De-winged samara soaked in water for 24 hr	1000	25	2.5	21
7	De-winged Samara soaked in water for 48 hr	1000	25	2.5	21

3.6.5. Seed storage and longevity

Seeds of *T. paniculata* were stored in gunny bags at room temperature for a period up to next seed collection and periodical viability tests were conducted with an interval of one month. Assessed seed longevity in terms of periodical germination trials and the result is depicted in Figure 10. A gradual decrease in seed viability was noticed under storage (from 2.6% of fresh seeds (5.9% MC to 0.2% at six months after storage (3.5% MC). The study revealed that seeds of *T. paniculata* shall be stored up to a period of 6 months. However, seeds should be sown within one month after seed collection at the optimum maturity stage for getting better germination. Various storage conditions were standardized for

other species in an earlier study as seeds in earthen pot/cotton bag kept inside wet vermiculite/saw-dust at 16 - 20°C and 45% relative humidity for *Artocarpus hirsutus*, *Calophyllum inophyllum*, *Syzygium cumini*, *Dysoxylum malabaricum*, *Gluta travancorica*, etc. (Pillai & Pandalai, 2015). Seeds of *Dendrocalamus brandisii* and *D. sikkimensis* with lower moisture content (8%) retained viability for 36 months under the storage condition of 4 ^{o}C and 45 % RH (Pillai *et al*., 2015),

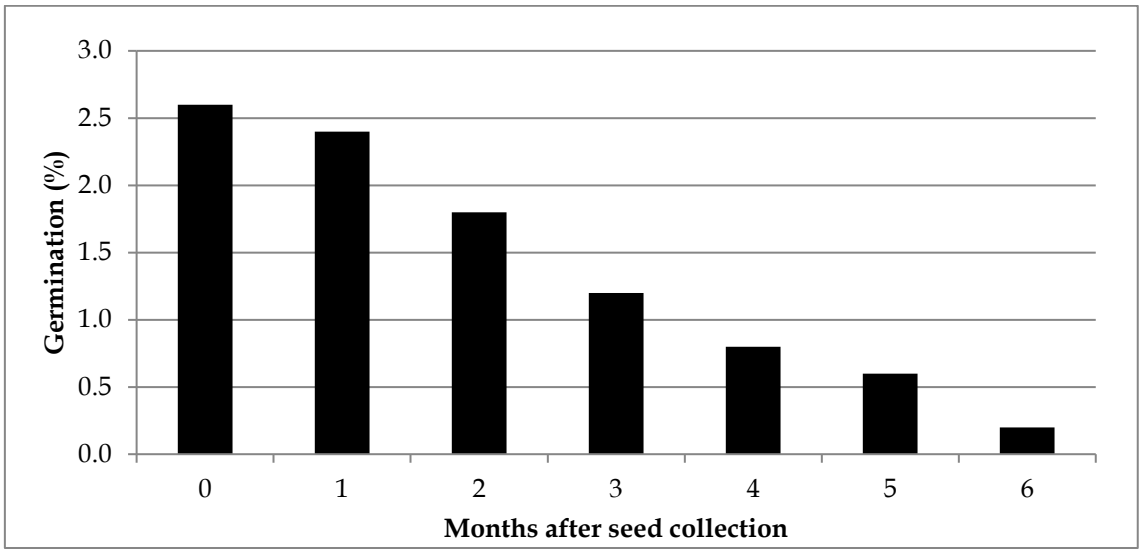

Fig. 10. Germination pattern of stored seeds at different storage period.

3.6.6. Regeneration

Status of mature trees of *T. paniculata* and its regeneration under *in situ* condition in Anamooli (Mannarkkad Forest Division), Nedumkayam (Nilambur South Forest Division) and Peechi (Peechi-Vazhani Wildlife Sanctuary) was assessed using the enumeration data collected from the study sites. Enumeration was carried out in temporary sample plots (50 m x 50 m) from the three locations and overall status were estimated and presented in Tables 4 and 5. All the mature trees were flowered except in the study site at Anamooli, where flowering and fruiting was noticed only about 50% of the mature trees (Table 4). This might be due to the presence of lower age group in the population. Overall tree density (number of trees per ha) in the study sites was about 97 trees ha^{-1}and flowering and fruiting status was in 80 trees ha^{-1} (Table 4).

Table 4. Status of mature trees in study sites (Values (individual ha^{-1}) are mean ± SD)

Location	Density (no. of trees per ha)	Height (m)		Girth (cm)		Flowering & fruiting status (trees per ha)
		Range	Mean	Range	Mean	
Anamooli	120	10 – 22	16.40	35 – 134	80.93	68
Nedumkayam	64	25 – 38	33.00	224 –350	299.98	64
Peechi	108	8 – 33	20.37	80 – 382	174.00	108
Overall mean	**97.33 ± 29.48**		**23.26 ± 8.67**		**184.97 ± 109.94**	**80.00 ± 24.33**

Regeneration status of *T. paniculata* in the study sites is presented in Table 5. The overall regeneration of the species in the study sites was 24.15/ha. Generally, about 52 per cent of the regeneration was under unestablished (seedlings) category (<3 cm girth class), 30% belonging to the established (saplings) category (3-9.9 cm girth class) and 18% in advanced (poles) category (10-30 cm girth class). About 45% of the unestablished categories were less than 50 cm height class, 46% were in 50-100 cm height class and 9% were above 100 cm height class.

Table 5. Regeneration status in study sites (Values (individual ha^{-1}) are mean ± SE)

Location	Regeneration categories					
	Seedlings (<3cm collar girth)			Total (< 3 cm collar girth)	Saplings (3.0 -9.9 cm GBH)	Poles (10-30 cm GBH)
	< 50 cm height	50-100 cm height	>100 cm height			
Anamooli	3.26 ± 0.68	6.38 ± 2.87	1.28 ± 0.58	10.92 ± 5.16	0.43 ± 0.18	0.28 ± 0.09
Nedumkayam	9.32 ± 3.27	4.70 ± 1.43	0.96 ± 0.21	14.98 ± 6.83	1.67 ± 0.53	1.20 ± 0.50
Peechi	4.57 ± 1.75	6.17 ± 2.63	1.17 ± 0.41	11.91 ± 4.37	19.79 ± 7.89	11.28 ± 5.88
Overall mean	**5.72 ± 3.19**	**5.75 ± 0.91**	**1.14 ± 0.16**	**12.60 ± 5.45**	**7.30 ± 2.89**	**4.25 ± 2.16**

According to Bhadra and Dhal (2010), density values of regeneration are considered as regeneration potential of the species. A good regeneration potential shows suitability of the species to the environment. Replacement of older trees in a forest by younger ones is an important process in natural forest maturation. Sustained timber yield and productivity from a forest largely depends on well-distributed age classes. A sustained yield forest can be even-aged, uneven-aged or a combination of both. Studies related to this field will contribute in planning, conservation and decision making in natural forest resource management. Natural regeneration is important as it addresses mainstream biodiversity concerns (Reddy & Ugle, 2008). Demographic assessment of regeneration of a forest type is useful to identify the constraints affecting natural regeneration.

Regeneration is important as it addresses mainstream biodiversity concerns and quantitative assessment of regeneration of tree species in a forest helps to predict future status of concern (Reddy & Ugle, 2008; Bhadra & Dhal, 2010). Within a primary forest, occurrence of individuals of a species in any particular spot is determined by the regeneration of that species, and it is governed by the presence of mature trees, dispersal mechanism, flowering and fruiting behaviour (Kartawinata, 1978; Menon, 2010). Nature, extent and pattern of over-storey vegetation offer distinctive resource supply regimes in forest under-storey. Survival and growth of regeneration depends invariably upon efficient utilization of these resources (Singh, 2003). Regeneration status of a species is determined based on population size of seedlings and saplings (Bhuyan *et al*., 2003). According to Khumbongmayum *et al*. (2006), regeneration is said to be good if the proportion is seedlings > saplings > adults, regeneration is fair if seedlings > or ≤ saplings > adults and regeneration is poor if the species survives only in sapling stage (saplings may be <, > or = adults). They stated that the future community structure and regeneration status of a species could be

predicted from the relative proportion of seedlings and saplings in the total populations of various species in the forest. Pokhriyal *et al*. (2010) also stated that regeneration of a particular species is poor if seedlings and saplings are less than the mature trees.

The study indicated that overall regeneration of *T. paniculata* in the study sites was good (seedlings > saplings > poles). However, the regeneration is not in par with mature/mother trees. Reddy and Ugle (2008) mentioned that reduced regeneration may be a threat to the species and the population structure will be unstable and regeneration potential will be negligible if the species is represented only by adults in any forest.

4. CONCLUSIONS

The study aimed to understand the reproductive constraints and develop seed handling techniques of *T. paniculata*. Vegetative as well as reproductive phenological events were recorded. Pollination biology, reproductive capacity, seed characteristics and regeneration patterns of the species were studied.

4.1. Vegetative and reproductive phenophases

Mature leaves of *T. paniculata* were observed throughout the year. Flushing commenced along with the South-West monsoon (June-September) and leaf maturation continued during the period and completed with the North-East monsoon (October-November). Flowering completed within six months (September-February) and fruiting in five months (December-April) before South-West monsoon. The study showed that the vegetative phenological events continued for six months (June to November); whereas, reproductive phenophase was for eight months (September to April).

4.2. Pollination Biology

Blooming was during 01.30 hr to 08.30 hr: opening of calyx in night (01.30 to 02.30 hr), erection of first 2 stamens in next one hour (02.30 to 03.30 hr), erecting other 6 stamens during 05.30 to 6.30 hr followed by the last 2 stamens during 07.30 to 08.30 hr. Anthesis was between dawns of first day to night of next day as 09.30-10.30 (Day 1), 16.30-17.30 (Day 1) and 03.30-04.30 (Day 2).

Even though massive flowering, pollinators were limited to nine species including night-active moths. Low rate of viable fruit production might be due to lack of sufficient pollinators. Breeding experiments resulted that cross pollination (xenogamy and geitonogamy) was successful in *T. paniculata*.

Pollen production was estimated as 16540±685.9 per flower with pollen-ovule ratio of 16540:1. The study found that blooming of flowers completed within a period of 6 hours and anthesis in 19 hours. Study on pollen germination and viability confirmed the pollens were viable for a period of 22 hours.

4.3. Reproductive capacity

Though about 28 per cent of the flower bud primordia developed into ripen fruit stage, only 1.32 per cent of them were produced viable seeds. Mean seed germination rate was 1.4 per cent. Average fruit production in a tree was estimated as 106080±47039 with average seed output (1400) and reproductive capacity of a tree was calculated as average seed output multiplied by average germination percentage (19.6).

4.4. Seed Characteristics

Fruit is a three-winged samara and the number of seeds per kg is 11,909 - 28,908. Optimum maturation period was between 11 - 25th weeks after fruit initiation and maximum viable seeds will be available at 16th week with red-coloured samara. Seed viability at 11th week after fruit initiation was 0.6 per cent and increased to 2.6 per cent at 16th week with 5.9 per cent moisture content. Thereafter, gradually decreased to zero per cent at 25th week with moisture content below 3.5%. Seeds harvested during 16th week can be stored in cotton bags at 4^{o}C up to 6 months without losing viability.

4.5. Regeneration

Overall regeneration density of *T. paniculata* in the study sites was 24.15 seedlings per ha. Though overall regeneration of the species in the study sites was good (seedlings > saplings > poles), they are not at par with mature trees and the reduced regeneration may be a threat to the species.

Compared to other species belonging to the genus *Terminalia*, *T. paniculata* showed low germinability, high seed emptiness and poor regeneration status. Even though the species produce massive flowers with viable pollen grains, the flowers were failed to develop viable seeds. The study concluded that artificial pollination between trees and harvesting of fruits at 16th week after fruit setting with red-coloured fruits are the suitable conditions to obtain viable seeds, which favours regeneration and thus helps to conserve the species.

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