Article

Seed bank estimation and regeneration studies of *Calophyllum apetalum* Willd., from Western Ghats of Karnataka

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Abstract

Studies on the seed production, modes of dispersal and regeneration patterns of threatened tree species are crucial for the management of their genetic diversity. The seed bank estimation of *Calophyllum apetalum* was assessed from three different locations of Western Ghats of Karnataka, using two factorial completely randomized design. The results revealed the seeds are dispersed by hydrochory and mammalochory. The post seed dispersal, seed bank estimation studies yielded a high seed density near the base of tree trunks, but it was varied between the distances and locations. The results revealed seed densities are insignificant among the forest ranges and significant with the distances. The *in-situ* regeneration studies revealed an insignificant relationship between the mean regeneration among the forest ranges and the distances. Also, the *ex-situ* regeneration studies resulted an insignificant relationship among forest ranges and the distances from which the seeds were collected. The highest seed germination through *ex-situ* regeneration suggested it, as a best suitable method of conservation of this species.

Keywords seed bank; *In-situ* regeneration; *Ex-situ* regeneration; conservation.

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1 Introduction

The seed dispersal in tropical rain forests is mediated by several biotic and abiotic agents involving successful establishment of new individuals at various distances from the mother trees. The relative importance of seed bank and regeneration process depends on several factors including the fruit or seed production, abundance and behavior of seed dispersers, and canopy gap characteristics (Castillo and Stevenson, 2010). Factors including site related traits are attracting seed dispersers in tropical regions in the presence of food resources such as fruits (Wunderle, 1997). The number of seeds dispersed from the trees decreases with distances from the parent trees (Barik et al., 1996). Rates of seed predation are influenced by conditions that alter seed supply and the activity of seed predators (Meeson et al., 2002). Climate change, alien species, and use of land for

intensive farming and development are causing severe threat to the plant genetic diversity worldwide. Hence, conservation of biodiversity is considered fundamental and also provides the livelihoods to millions of people worldwide (Krishnan et al., 2011). Although the rich floral and faunal diversity of India has been established beyond doubt, much remains to be done in the area of conservation of biodiversity and eco-restoration (Rao et al., 2009). Areas of high conservation value were identified in the Western Ghats using a systematic conservation planning approach (Das et al., 2006). With the increased loss of plant biodiversity, the *ex-situ* seed conservation has become of strategic importance (Rossi et al., 2014). Genetic resources conservation has been less than completely satisfactory over time as a result of weak or poorly funded *ex-situ* collections while *in-situ* efforts have been virtually nil. Users of plant genetic resources are accustomed to working with *ex-situ* gene bank curators and databases in selecting genetic materials for plant research (Shands, 1991, Li et al., 2002). The studies on pollination and reproductive biology are also play an important role, as they possess direct influence on seed output (Shivaprasad et al., 2015). The studies on pollination and reproductive biology of threatened tree species from Western Ghats are few but, they are valuable as they give basic notions for the further management and conservation of such species (Shivaprassad et al., 2014, Kumar et al., 2014a). *Ex-situ* approaches, in contrast, have been relegated to a subsidiary role and their direct contributions to conservation have been limited (Pritchard et al., 2012). Maintaining living *ex-situ* collections is one of the key conservation methods in botanic gardens worldwide.

The genus *Calophyllum* belongs to the family Clusiaceae, consists of about 130 species, out of which seven are reported from India (Joshi et al., 2013). *Calophyllum apetalum* Willd., is a multipurpose evergreen tall tree, endemic to the Western Ghats of Southern India (Nair and Seeni, 2003), distributed mainly along the streams and river banks with an elevation range up to 1300 m (CSIR, 1992). Different parts of this plant are used in the traditional system of medicine for the treatment of several diseases (Joshi et al., 2013). The seed oil is reported to possess antiseptic properties and is used in the treatment of rheumatism, leprosy, scabies and other cutaneous afflictions while the leaf juice is used for gastric problem (CSIR, 1992). The stem bark exudes a translucent resinous fluid used as vulnerary, resolutive and anodyne (Chopra et al., 1956). Oil extracted from the seeds possess antiseptic properties and is used for treating rheumatism, scabies and other cutaneous infections and as a septic poison (CSIR, 1992). The chemical examination of different parts of *C. apetalum* led to the isolation of xanthones and tri-terpenoids (Govindachari et al., 1967, Xiao et al., 2008, Iinuma et al., 1997). Few workers attempted to rise the seedlings of this species, in the disturbed sites within the Western Ghats under the tree canopies of introduced plantations showed their survivability for long duration (Nagaraja et al., 2001b, Nagaraja et al., 2001a). Studies on pollination and reproductive biology of the species reported it as a self-compatible, pollinated with mellitophily and catheranthophily with a highest fruit set recorded over the manual cross pollination experiments (Kumar et al., 2014b). This self-compatible species can often produce seeds when pollinators are scarce or unreliable, but any advantage may be lessened if selfed progeny are less fit than outcrossed progeny due to inbreeding depression (Vaughton and Ramsey, 2006). The current study conducted at the selected sites of Western Ghats region to assessing the modes of seed dispersal, seed bank densities and regeneration through both *in-situ* and *ex-situ* studies. The results are useful for the further management and conservation of this threatened species.

2 Materials and Methods

2.1 Fruit/seed dispersal mechanisms and regeneration studies

The seed bank density at different distance of seed dispersal was studied by selecting five trees each from three different locations using *two factorial completely randomized design*. The plots were established around each mother tree along four directions. Seed dispersal and regeneration plots with a dimension *1mX1m* were

established at a distance of approximately 5, 10, 15, 20 and 25 meters from the edge of tree canopy followed by enumerating the number of seeds and regenerated seedlings. The average seed density from each fixed distance was calculated from the four values. The significant difference in the mean percent of seed density, seedling regeneration from each distance was compared between the sites using critical difference (CD) and *F*-table values obtained from the analysis. The mean percent of natural regeneration from the random plots were calculated for each distance from the base of the mother tree as their weighed average using the formula,

$$\text{Mean percentage (\%) of germination} = \frac{\text{Average number of seeds germinated}}{\text{Average seed count of all the plots}} \times 100$$

Trees with diameter at breast height (DBH) above 10 cm were located and selected from three locations from the Western Ghats regions to assess the distance of seed dispersal and the regeneration patterns includes *in-situ* and *ex-situ*. The locations selected several forest ranges such as Agumbe, Kukke-Subramanya, and Uppinangadi (Gundya) forest ranges. The data on fruit/seed density from the base of the tree at a distance of 5m, 10m, 15m, 20m and 25m were measured. The data were analyzed using *two factors randomized complete block design*. Three '*forest ranges'* in each case served as a *first factor 'A'* and '*distances*' of the plots from the canopy edge of each mother tree as a *second factor 'B'* in the design.

2.2 Regeneration studies

Five individual mother trees were selected from each locations and random quadrates of 1m X 1m, were laid in such a way that each plot should be at least 5m apart and the distance between the mother trees to each plot was measured. The number of fruits per each quadrat was counted each day during the fruit ripening and dispersal. The observations were continued until regeneration. The regeneration success was monitored by counting the number of seedlings (Kuuluvainen and Kalmari, 2003).

2.3 Experimental design: Two factors completely randomized design

The significant effect of *seed density* and *regeneration* between distances and locations of each species was determined by using a *completely randomized two factor design*. The factor *A* with '3' levels (locations, *i*) and factor *B* with '5' levels (distances, *j*) with *three* replications each per factor combination, were taken to reduce the experimental error. The significant effects of two factors viz., locations (factor A) and distance (factor B) and their interaction effects (A*B=45 levels) were calculated by computing the Fisher's '*F*' value and *critical difference* (CD) values at *95%* confidence intervals. The following *hypothesis* were tested.

3 Results

The seeds are dispersed by anemochory (wind), hydrochory (water), and mammalochory (mammals). The seeds are rich in essential oils and nutrients. Mammals involved in seed dispersal include porcupine (Hystricomorph sp.), barking deer (*Muntiacini sp*.), Giant squirrel (*Ratufa indica*), rodents, Sambar (*Rusa unicolour*) and spotted deer (*Axis axis*) the seeds are dispersed through these fauna but, most of the seeds are predated by them before successful dispersal in viable form.

Although the distance of dispersal by anemochory is observed to be up to 100m from the tree canopy, the seeds are also dispersed up to several kilometers through hydrochory. The germinated seedlings are established after post-dispersal on the streams and river banks within about 10-15 days. The natural seed germination was good enough to form a stable population size. The seeds dispersed alongside the river path are more prone to decay or wash out during heavy rainfall. The seedlings germinated along the river way also face a similar problem. Successfully established germinated seeds develop and establish as saplings after 2-5 years. Nonetheless, about 1-2% of the total seed output established into mature trees.

The post seed dispersal seed bank estimation studies yielded a high seed density near the base of the tree, but it varied between the distance and the locations. The highest mean seed density per square meter was 39.01±0.72, 33.65±1.41, 35.91±0.63 at a distance of 5m, 10m and 5m and the lowest mean seed density per square meter is 4.20±0.3, 4.75±0.9, and 10.83±1.46 at a distance of 25m from Agumbe, Gundya and Kukke-Subramanya respectively (Fig. 1).

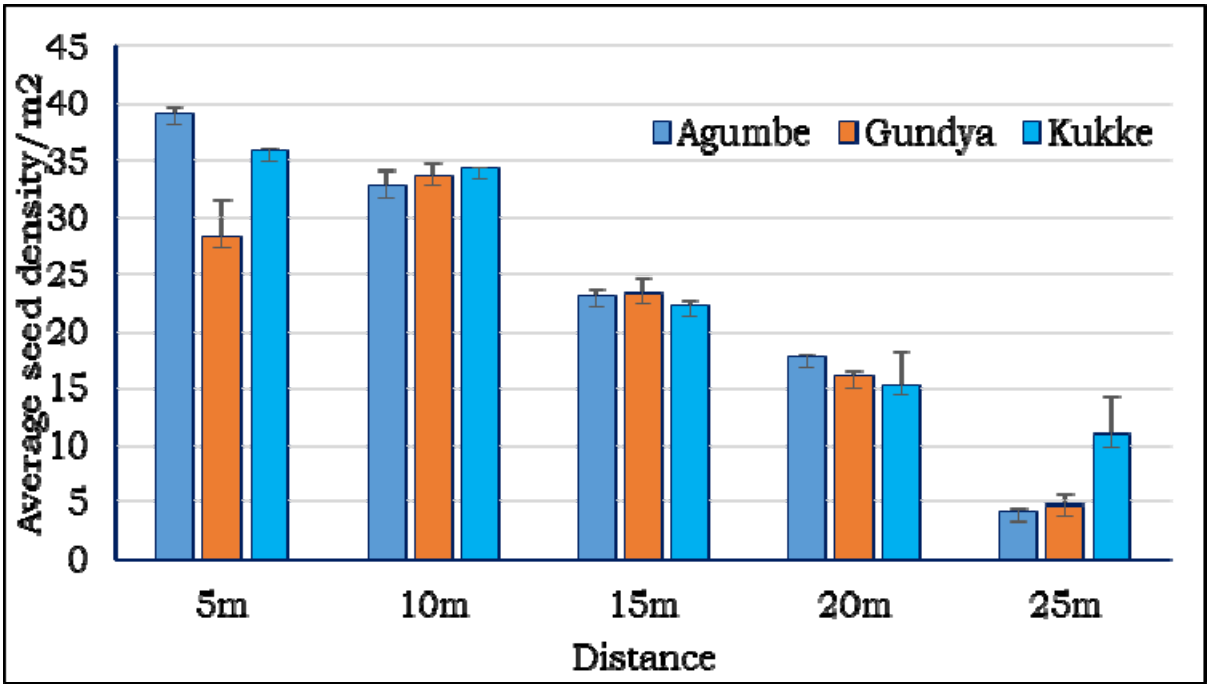

Fig. 1 Seed bank estimation studies on *C. apetalum*.

The results revealed that there is no significant difference with the seed densities among the forest ranges ($F_{0.95}$, $p=0.4$). Similarly, there is significance with the seed densities among the distances ($F_{16.82}$, $p=0.01$). The interaction effect of forest ranges and distances are statistically significant ($F_{8.92}$, $p=0.03$).

The *in-situ* studies resulted in a higher mean percent of regeneration of 86.52±0.28, 87.15±0.01, 81.06±0.08 at a distance of 25, 5 and 20m in Agumbe, Gundya and Kukke-Subramanya respectively. The lowest mean percent of regeneration is 14.48±2.46, 37.20±4.16, and 46.80±2.88% at a distance of 25m, 5m and 20m from Agumbe, Gundya and Kukke-Subramanya respectively (Fig. 2).

The results revealed that an insignificant relationship between the mean *in-situ* regeneration among the forest ranges ($F_{0.74}$, $p=0.53$). So also the relationship of *in-situ* regeneration among the distances is insignificant ($F_{12.37}$, $p=0.06$). Even interaction effect of forest ranges and the distances on *in-situ* regeneration is statistically insignificant ($F_{6.31}$, $p=0.056$).

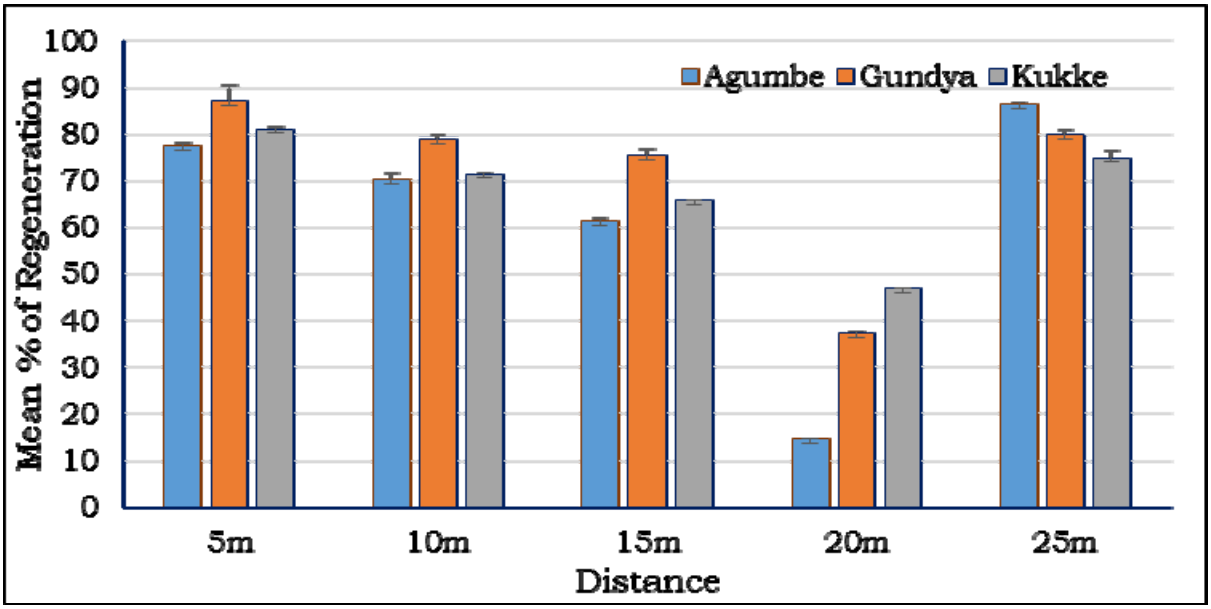

Fig. 2 *In-situ* regeneration studies on *C. apetalum*.

The *ex-situ* regeneration studies resulted in a higher mean percent of seed germination of 98.89±1.95, 98.89±0.92, 99.99±0.01% at a distance of 25m, 15m and 15m and the lowest mean percent of regeneration is 94.44±1.92, 95.56±4.16, 95.56±0.85% at a distance of 10m, 20m and 10m from Agumbe, Gundya and Kukke-Subramanya respectively (Fig. 3).

The results revealed that an insignificant relationship between the mean *ex-situ* seed germination among the forest ranges ($F_{0.12}$, $p=0.80$). This remained insignificant among the different distances ($F_{1.19}$, $p=0.83$). Also, the interaction effect of forest ranges and distances on the *ex-situ* regeneration also remained statistically insignificant ($F_{1.16}$, $p=0.8$).

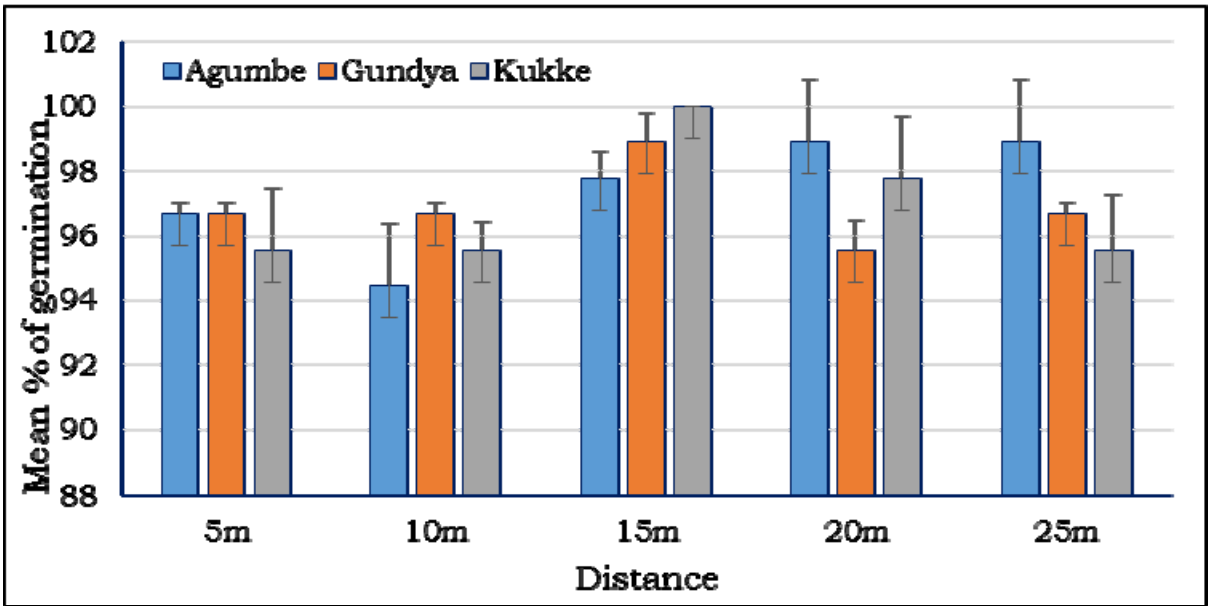

Fig. 3 *Ex-situ* regeneration studies on *C. apetalum*.

4 Discussion

Two factor analysis of seed dispersal studies revealed an insignificant effect of locations and distances, implying that the dispersal is uniform across the locations. However, their interaction effect was significant, suggesting the involvement of factors that influence seed density at each distance, including the canopy width, animal dispersers, wind speed, water flow, slopes etc. The results on *in-situ* regeneration showed a good mean percentage of seedlings regenerating across all the sites and they are insignificant, suggesting high seed viability and the ability to regenerate. The interaction effects of locations and the distances are insignificant implying that the regeneration is independent of the locations and distances in case microclimate such as soil moisture, nutrient availability are ensured. The *ex-situ* regeneration studies showed an insignificant relationship between the forest ranges and distances revealed that the seeds are collected from all the locations yielded a high mean percentage of seed germination suggesting high quality of seeds across all the three sites.

The anthropogenic disturbances, including the chopping of trees for timber extraction, lopping branches from saplings and small trees for making organic manure are also posing a major threat. The seed predation on the other hand is helping partial seed dispersal, but continuous removal of most of the seeds from the forest floor is directly affecting successful regeneration and establishment. These problems can be overcome through *ex-situ* regeneration and by maintaining the saplings in nursery and, later reintroducing them to forest as an effective method to restore the population.

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