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In vitro and in vivo seed germination and phytosociology of an endemic and critically endangered species, *Hildegardia populifolia* Roxb. of Malvaceae, from Tamil Nadu

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ABSTRACT

Hildegardia populifolia (Roxb.) Schott & Endl. is a deciduous, endemic and critically endangered tree of Malvaceae. It is disjunctly distributed at the south eastern ghats between the two states, Tamil Nadu and Andhra Pradesh. During the population assessment, 45 quadrats with 10 x 10 m2 have randomly selected at *H. populifolia* habituated areas in Pakkam Malai reserve forest, near Gingee, Viluppuram district of Tamil Nadu, found that the regeneration status was inferior. To know the seed viability and germination potential, this study has planned to make an easy and efficient germination protocol for mass multiplication and to conservation programs. Tested by three traditional methods, viz. 1. seed coat scratch method, 2. water-soaked method and 3. cow dung water-soaked method. The second method shows better germination (90%) results than the other two methods. Another study, found 40% of germination after 25 days from the aseptic naked seeds inoculated in culture medium. The protocol developed here would help to execute large-scale ex situ conservation programs for this endangered species. Field observation and the lab condition indicated that the fungus *Penicillium citrinum* inhibits the growth of germination.

Keywords: Conservation; Gingee hills; *Hildegardia; Pencillium,* Sterculiaceae; Viluppuram.

1. INTRODUCTION

Hildegardia belongs to the family Malvaceae (earlier Sterculiaceae) and sub-family Sterculioideae (Angiosperm Phylogeny Group, 2016). *H. populifolia* species was first recorded by Heyne in 1800 from the Coromandel coast, without citing any specific locality. Later Roxburgh, (1832) made the first valid publication in Flora Indica.

Hutchinson, (1967) has distinguished this genus from the other genera of Sterculiaceae by its flowers lacking petals and epicalyx, possessing androgynophore and indehiscent thin membranous or papery fruits. He also stated, this genus consists of 12 species and has pantropical distribution: West Africa, East Africa, Madagascar, southern India, Philippines, Indonesia, northern Australia and Cuba. Zaborsky, (2009) noted that three of them (*H. barteri, H. gillettii*, *H. migeodii*) are found in Africa, four (*H. ankaranensis, H. erythrosiphon, H. perrieri, H. dauphinensis*) in Madagascar, one (*H. australiensis*) in Australia, one (*H. cubensis*) in Cuba, and three (*H. merrittii, H. populifolia* and *H. sundaica*) in Asia. Of which *H. populifolia* is the only species that occurs in India.

In Tamil Nadu, Matthew, (1983), Nair and Henry, (1983) and Ahmedullah, (1990) have recorded the occurrence of this taxon from Kalrayans, Gingee and Tiruvannamalai (personal obs. of the first author) hills from Kallakuruchi, Viluppuram and Tiruvannamalai districts of Tamil Nadu respectively. Meanwhile, Jayaprada, (1998), Pullaiah and Ramamurthy, (2007), Murthy et al., (2007), Rao et al., (2011), were recorded this species from Chittoor, Anantapur, Guntur and Kadapa districts of Andhra Pradesh. The conservation status of *H. populifolia* species has been assessed differently as "*Critically Endangered*" Sarcar and Sarcar, (2002), "*Endangered*" Ahmedullah, (1990), Walter and Gillet, (1998), Rao et al., (1998) and "*Vulnerable*" (Reddy et al., 2001).

It is locally known as "Malai puvarasu" in Tamil; which is endemic to Eastern Ghats and disjunctly distributed on the slopes of Andhra Pradesh and Tamil Nadu, southern India. It is a drought resistant and deciduous tree, grow 12-15 m high, girth 2-3 m with smooth bark, from poor stony soils into the steep, rocky and dry slopes. During the dry season, the entire tree undergoes significant phenological changes. Leaves cordate, 6.5-10 x 5.5-10 cm falls between January and March. A new flush followed by flowering occurs during May and June. Inflorescence a terminal panicle, flowers polygamous. The fruit is 5-follicles with one or two seeds in every follicle and generally sheds on the rocky floor (Figure 1). Seeds with a hard coat, it may or may not germinate readily during the rainy season due to the physio-chemical responses of the micro-habitat.

In the field, we observed that one *H. populifolia* tree produces many seeds; earlier studies revealed that propagation through seeds also easy; it has 2-3 months viability but it is unknown, why most of the seeds fail to germinate in natural condition. Generally, the seeds of *H. populifolia* were not buried in the soil but stored on the floor or a rocky surface; man-made fires are mostly lethal to them especially when the fuel load is high. Partially burned seeds have germinated and reached seedlings stage but their further growth is depending on the availability of water, nutrient content and climatic conditions of the soil.

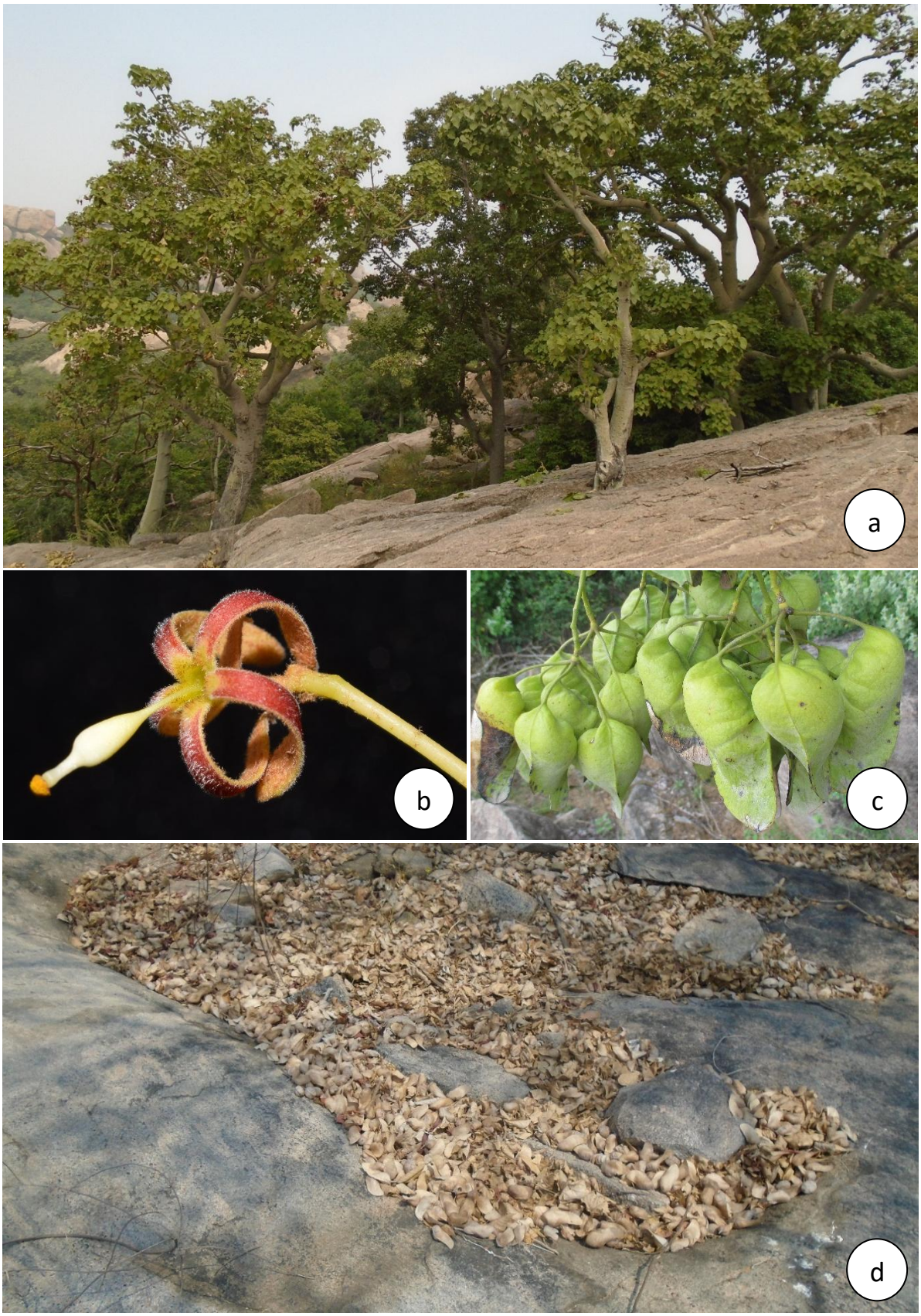

Figure 1 Habit, habitat, and phenology of *H. populifolia*.

2. MATERIALS AND METHODS

Study Area

Gingee (or Senji) is one out of eight taluks in the Viluppuram district of Tamil Nadu; famous its fort but have a chain of 8 reserved forests, of which Pakkam Malai reserve forest (12° 10′52.43″ N, 79° 18′12.71″ E, 353 asl) is one among them, has studied. According to Champion and Seth, (1968) classification there are three major forest types found here: viz., the Tropical Dry Evergreen Forest (TDEF), Tropical Dry Evergreen Scrub (TDES) and Dry deciduous (DD) (Figure 2). According to Meher-Homji and Misra, (1970), the geological formation of Villupuram district had happened during Miocene period. The soil along the coast is sandy loam or red ferralitic, in certain places, covered with alluvial deposits, becoming clayey in the interior (Meher-Homji, 1974). The substratum has been erupted into hillocks and mounds rise to 450 m elevation with interrupted vegetation among the charkonite or gneiss rocks.

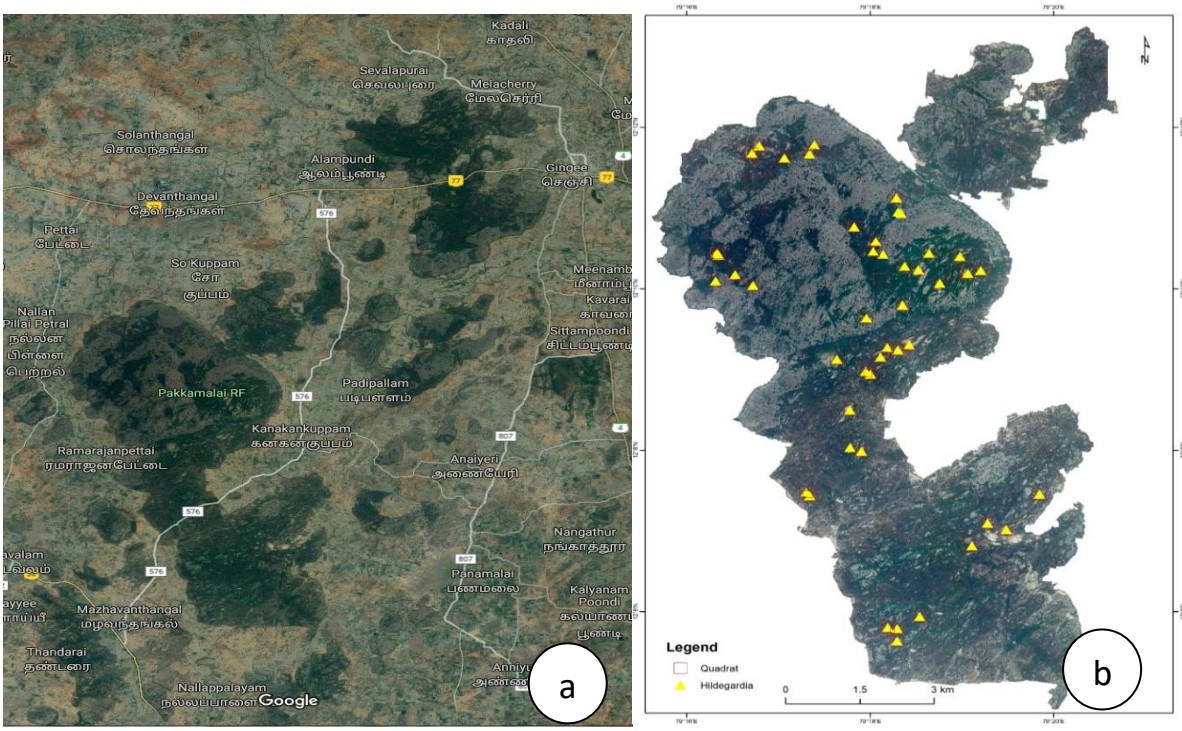

Figure 2 Study area: Pakkam Malai RF, Villupuram districts of Tamil Nadu with quadrat studied plots earmarked.

A typical maritime tropical climate with a dissymmetric rainfall regime, hot and humid weather for most of the year with minor variations occurs in the study area. The South-West monsoon have contributed 18 to 23% of total annual rainfall during June-August months and 60 to 95% of rainfall during North-East monsoon which occurs between the months of September-December (Meher-Homji, 1974). The mean annual rainfall is 1,256 mm, with mean rainy days of 56 per year. The minimum temperature is 17.7°C is in January, the maximum temperature 40.5°C in May and the mean is 28.5°C. The average relative humidity is 76%. The weather is generally cool from December to January and the dry weather prevails from April to June. Wind speed ranges from 5.0 km/h between June and July to 9 km/h between August and September but significantly higher during the cyclonic days.

Seed germination

During January healthy follicles with seeds were collected from matured individuals to raise the seedlings in two different methods: *viz*., traditional and in vitro.

Traditional Methods

There are three kinds of methods followed between January and March: viz., seed coat scratched, seeds soaked 24 hours in water and seeds soaked 24 hours in cow dung water. These treated seeds were sown in a pot contains the mixture of farmyard manure, red earth

and soil and it receives water every alternate day. In each test, three experiment pots and one control pot were maintained. In every pot, five seeds sown *i.e*. a total of 15 seeds were sown. All these pots were kept in a controlled environment.

In-Vitro seed germination

Healthy seeds were treated by soaking the seeds in Tween 20 for 10 min and rinsing with tap water for 10 min. The scarified seeds have disinfected in 70% ethyl alcohol for 1 min followed by 0.1% Mercury Chloride solution for 10 min. Then, the seeds were rinsed 4-5 times in sterile distilled water. Sterilized seeds were inoculated in basal MS medium. The inoculated seeds were incubated under the optimal culture conditions. The seedlings raised through in vitro method have transferred to earthen pots containing soil and sand (1:1), and these have maintained in the garden at room temperature (25 ± 2◦ C). The survival rate has recorded every 20, 30 and 40th days and then the seedlings were transferred to the field environment.

3. OBSERVATION AND RESULTS

From the field

About 45, 10 × 10m2 quadrats were randomly selected and studied; in which we found only ten seedlings whose girth <10 cm at breast height were recorded and the saplings were also suffering due to prolonged dry period and erratic rainfall. In all, the natural regeneration of the species through seeds is very poor in the field condition. From the regular survey at Pakkam Malai RF, we observed that thin sheath of epicarp gradually disintegrates and exposes the seeds between March and April. After few summer rain the seed germination is dependent on the disintegration of the hard and thick seed coat and these are mainly depending on the micro habitat of the area, especially with the litter load and the moisture content during germination.

This Critically Endangered species has been associated with 40 (51.3%) deciduous and 38 (48.7%) evergreen woody plants. *Gyrocarpus americanus*, is a deciduous species commonly associated, and other main associates are *Barleria longifolia, Cissus arnottiana, Combretum ovalifolium, Commiphora caudata, Euphorbia antiquorum, Ficus mollis, Givotia rottleriformis, Grewia flavescens*, *Hugonia serrata, Lannea coromandelica, Memecylon umbellatum, Phyllanthus pinnatus*, *Psydrax dicoccos, Spondias pinnata, Tarenna asiatica,* and *Ziziphus oenophlia.* Attempted traditional and *in vitro* germination methods to know the germination condition of the seeds.

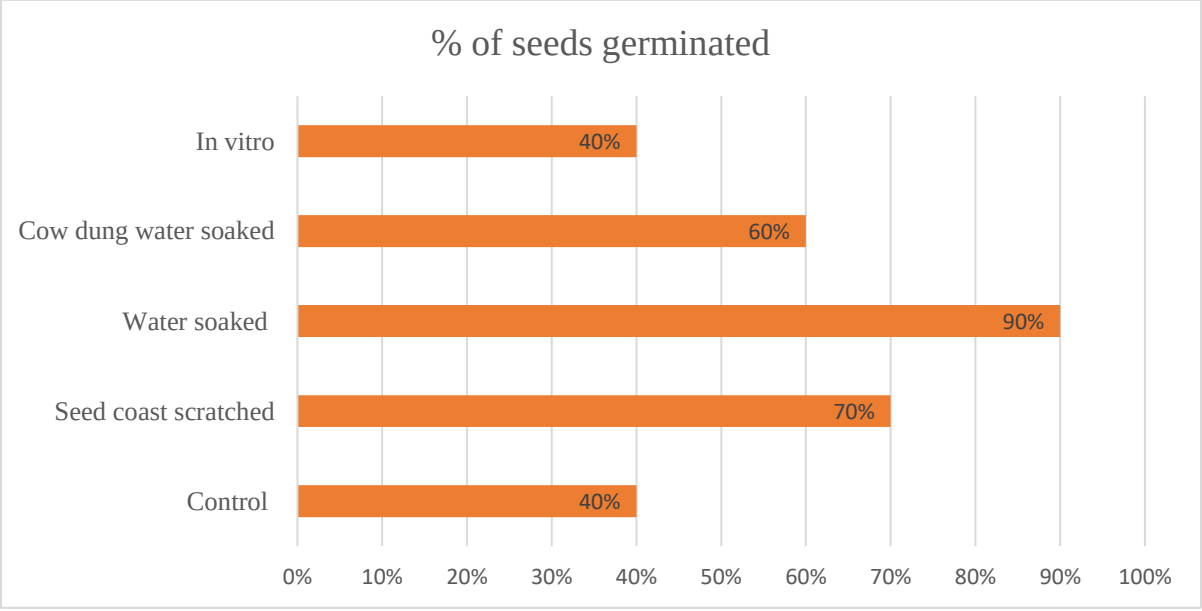

Figure 3 The results from traditional and in vitro seed germination methods of *H. populifolia*

In conventional methods, initial seed germination has observed on 13th day after the seeds were sown in the pots. The results are, 40% from control, 70% from scratched seed coat, 90% from water soaked and 60% from cow dung water-soaked test, whereas in culture medium only 40% of germination was recorded (Figure 3, 4, 5). Under in vitro conditions most of the seeds have inhibited by a fungus. Efforts were attempted to isolate the fungus, then cultured separately and identified as *Peniicillium citrinum* (Figure 6). Germinated seedlings have transferred after 35 days to individual plastic bags. After transplanting in the polybags, the seedlings have kept under 30 days of acclimatization. Later, the seedlings were planted in soil and we recorded 100% survival rate (Figure 5). The seedlings raised through lab conditions have placed in green house conditions for one month, in which 20 seedlings have planted at the institute campus and they are performing well.

Figure 4 Traditional methods of seed germination: a: Seeds, b: sown in earthen pots, c: Seedlings in cotyledonary stage, d: Seedlings with three leaves.

Figure 5 In vitro method of seed germination - planting; a: Inoculation, b: Germination; c: Hardening, d: Planting in a garden.

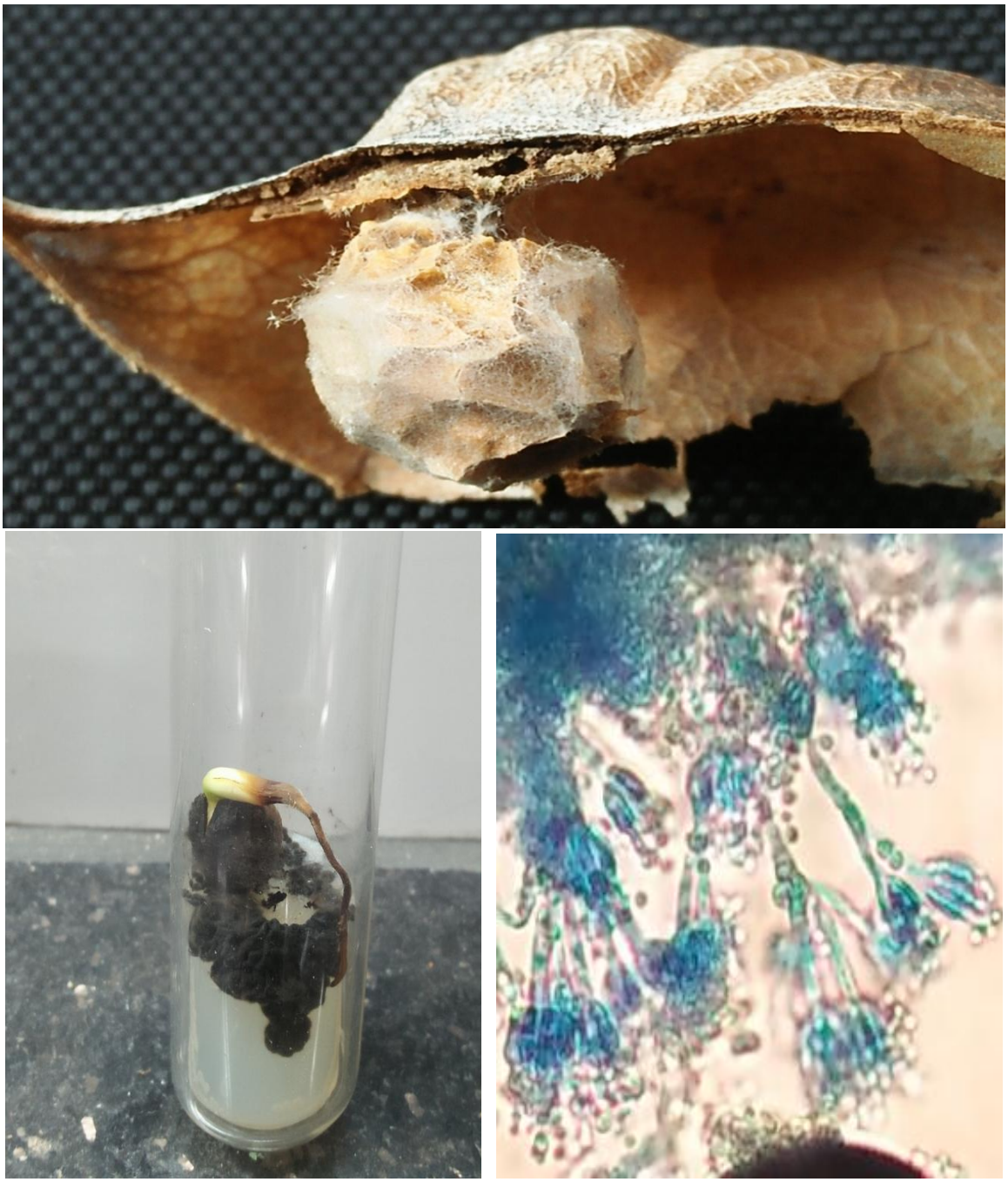

Figure 6 *Pencillium citrinum* infection on the seed coat and in the lab and its isolated form.

4. DISCUSSION

Hildegardia populifolia is one of the endemic and critically endangered deciduous trees of the Eastern Ghats of Tamil Nadu and Andhra Pradesh, Southern India. It is growing well in Pakkam Malai RF, Gingee hills of Viluppuram District, Tamil Nadu. Like *H. populifolia*, Pakkam Malai RF harbours quite interesting endemic and RET species. About 23 are disjunctly distributed between the districts, states, ghats, climatic regimes, and bioregions (Balachandran and Rajendiran, 2014; Balachandran et al., 2018). From four different seed germination methods, 40% of germination obtained from control test, 60% from cow dung-soaked test, 70% from seed coat scratch test and water-soaked test bagged the highest i.e. 90% was recorded. Thus, the later test recommended as the cheap and best method for mass propagation. In contrast, inoculation of naked seed in culture medium recorded only 40% of germination, in which most seeds have infected with the fungus, *Penicillium citrinum*, considered the critical causal agent inhibiting the regeneration in the field.

Meanwhile treated the seeds with sulphuric acid (H2SO4) by 5, 15, 30, 45 and 60 minutes have recorded 30, 45, 70, 85 and 98 % of germination respectively. She was also treated with Tetrazolium Chloride; the result was 100% positive. Another study by Anuradha and Pullaiah, (2001) reported callus induction from shoot explants of *H. populifolia* on MS+BA (2.0 mg/l) medium. Saradha and Paulsamy, (2015) has studied through mature stem cuttings is an effective propagation method than the seed germination. In contrast, stated the cutting method is not efficient under varied climatic conditions to repopulate this species in its pristine habitat. However, she found that micro-propagation between axillary and apical bud of *H. populifolia*, is an economical and efficient method for conservation programs.

Anuradha and Pullaiah, (2004) has reported the cotyledons and embryos got damaged during maturation stage due to fungus, which leads to number of viable seeds drastically reduced. It might be the reason for poor germination and survival rate of seedlings in natural conditions and the size of population reduction. This current work found one particular fungus growing in all propagation methods and inhibits the growth of seedlings. Further study has undertaken to isolate the fungus, cultured separately in the medium and identified as *Penicillium citrinum* (Figure 3). It is the primary causal agent that inhibit the seed germination in the field and lab. Anuradha and Pullaiah, (2004) also tested, 75-80% of germination recorded from the premature seeds but the babies failed to grow beyond two leaf stage. Saradha and Paulsamy, (2015) reported that clonal propagation through mature stem cuttings is more effective propagation method compared to the seed germination rate. However, stated that the cutting method is inefficient under varied climatic conditions to repopulate this species in its pristine habitat.

5. CONCLUSION

All *Hildegardia* species also experience the same regeneration problems since they are all deciduous, occur in rocky habitats, and have small populations or a few individuals in their natural habitats. The study suggests that the seedlings of *H. populifolia* should be raised in nurseries and then transferred to natural habitats to increase the population size of the species. The easiest and cheapest way of restoration method is to collect the potential seeds on time, store them in proper environment, and peg the seeds as much as possible in pleasant places, after couple of showers, to grow and establish. Further, appropriate measures to be taken for effective conservation and management for this endemic and critically endangered species will become extinct in the wild.

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Authors Contributions

Both authors conducted field survey, the first author made conceptual design, analysis and critical revision of the manuscript, and JP drafted the manuscript, and both authors read and approved the final version of the article.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Ethical approval & declaration

In this article, as per the plant regulations followed in the Ecology Department, French Institute of Pondicherry, India, the authors observed the seed germination and phytosociology of an endemic and critically endangered species, *Hildegardia populifolia* Roxb. of Malvaceae, from Tamil Nadu, India. The ethical guidelines for plants & plant materials are followed in the study for species collection & identification.

Data and materials availability

All data associated with this study are present in the paper.

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