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Development of seed handling technologies for selected bamboo species

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PROJECT PROPOSAL

Project No.	:	KFRI RP 624/2011
Title	:	Development of seed handling technologies for selected bamboo species
Principal Investigator	:	Dr. P.K. Chandrasekhara Pillai
Associate Investigators	:	Dr. K.K. Seethalakshmi (PI - till May 2013) Mr. V.P. Raveendran Dr. G.E. Mallikarjunaswamy
Research Fellow	:	Miss. C.J. Lakshmi
Objectives	:	1. To standardize methods for seed collection, processing and testing seeds of different bamboo species. 2. To develop storage methods for orthodox types of bamboo seeds. 3. To assess the factors affecting seed viability such as biochemical changes and infestation with fungi and insects.
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CONTENTS

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ABSTRACT

Investigation on seed handling technologies for two bamboo species, viz., *Dendrocalamus brandisii* and *D. sikkimensis* was carried out. The species were selected considering their ecological and economic importance. Seeds of *D. brandisii* were collected from Ponnampet, Coorg District, Karnataka (12° 08' 32.5" N; 75° 55' 16.0" E) and *D. sikkimensis* from the Field Research Centre of KFRI, Velupadam (10° 26' 07.95" N; 76° 21' 32.92" E). Seed attributes such as seed weight, length, width, thickness, moisture content and physical purity were determined. Rapid viability tests (tetrazolium & hydrogen peroxide test) and germination test were carried out for assessing seed viability. Seeds were air-dried up to the lowest safe moisture level (8 %) for successful storage. Seeds of both the species were stored in polythene bags (0.05 mm thick) and kept under three conditions (4 °C, 16 °C & ambient temperature) to standardize the best storage condition. Periodical viability tests and moisture analysis were carried out to study the effect of storage condition, seed moisture variation and seed longevity. Seeds were sown in vermiculite, quartz sand, germination paper and soil to standardize the best germination medium. Accelerated ageing test was conducted to predict storability of seeds. Mycoflora associated with seeds under storage were also studied. Effect of leaf extract (*Azadirachta indica, Aegle marmelos* and *Lawsonia inermis*) and fungicides (bavistin, thiram and indofil) on seed mycoflora and seed deterioration were evaluated.

Seeds of *D. brandisii* and *D. sikkimensis*, with initial viability (60 & 52 % seed germination), when stored for 36 months at 4 °C and 45 % relative humidity (RH) maintained their viability throughout the storage period. Under ambient temperature (room temperature: about 32 °C) and 16 °C, the seeds were viable (60 % germination) only up to five and ten months for *D. brandisii*, and four and ten months (52 % germination) for *D. sikkimensis*. Seed moisture content significantly influenced seed viability and germination; an increase of moisture content from 8 -12 % resulted in complete loss of germination (0 %) for *D. brandisii* and *D. sikkimensis.* Seed germination was highest in vermiculite medium for *D. brandisii* (60 %) and *D. sikkimensis* (52 %), followed by quartz sand (55 & 48 %), germination paper (55 & 47 %) and soil (36 & 35 %) respectively.

During ageing, starch content in seeds were hydrolyzed by the activity of starch degrading enzymes such as α and β- amylases, resulting in decrease of starch content and increase in the activity of amylases. The decreased activity of phosphatases and peroxidases during ageing adversely affected metabolism of seeds resulted in deterioration of seeds and loss of viability. Increased total free aminoacids and the activity of amylases confirmed degradation of seed reserves while aging.

Thirteen fungal species were identified on stored seeds. Seeds stored at 4 ^{o}C and 45 % RH had minimum fungal incidence. Treatment with leaf extract of *A. indica* reduced the incidence of mycoflora from 14.83 to 0.08 % in *D.brandisii* and 17.37 to 0.11 % in *D.sikkimensis*. Similarly, seeds treated with indofil significantly reduced mycoflora from 14.83 to 0.06 % in *D.brandisii* and 17.37 to 0.06 % in *D.sikkimensis*.

Based on the study it is concluded that for improving shelf life of *D. brandisii* and *D. sikkimensis*, seeds shall be treated with *A. indica*/indofil and stored in double polythene bags in airtight plastic container at 4 ^{o}C and 45 % RH after drying to 8 % seed moisture content. Similarly, vermiculite is the best medium for germination of bamboo seeds.

1. INTRODUCTION

Bamboo is one of the most useful renewable natural resources and plays a significant role in the livelihood of rural people and rural industry. It has more than 3000 well documented uses for food, construction, shelter, bridges, household articles, agriculture, fisheries, transportation and village industry (Seethalakshmi & Kumar, 1998). India is the second largest diversity centre for bamboos with 18 genera and 128 species. Recent report revealed that 122 species belonging to 26 genera occur in India (Kumar *et al*., 2007).

Three types of flowering are reported in bamboos: annual, gregarious and sporadic flowering. Most of the commercially important bamboo species belong to the gregarious flowering group, where the flowering starts synchronously in all the daughter clumps originated from one parent clump and leads to the death of entire population after seed setting. Death is the result of reproductive exhaustion caused by movement of food reserves from the vegetative parts (Janzen, 1976). Flowering interval of gregariously flowering bamboos varies from 3 to 120 years (Janzen, 1976). Though, abundant seed production is observed during gregarious flowering, viability of seeds under natural condition is very poor. Conventional methods of vegetative propagation like rooting of culm cuttings and rhizomes have disadvantages such as limited availability, high cost, labour intensive, difficult to transport, requirement of larger nursery area, season bound and low percentage of rooting and synchronous flowering. Propagation through seeds is efficient and cost effective. Therefore, seed propagation is the most dependable source of planting stock provided seed is available. Since flowering cycle is long, it will be extremely useful to develop appropriate storage method that would prolong the viability of seeds so that planting stock can be raised in the subsequent years.

Bamboo fruits are generally known as Caryopsis. However, Wen and He (1989) classified seeds based on the morphology into three types: Caryopsis, Glans and Bacca. According to storage behavior seeds are grouped into three categories, *viz*., orthodox, recalcitrant and intermediate types (Roberts, 1972; Tompsett, 1984; Ellis *et al*., 1990). Based on this, method of storage varies with species.

One of the major limitations in bamboo propagation through seed is their shortest shelf life. Rapid decrease in seed viability and germination is the biochemical changes associated with seed ageing (Ravikumar *et al*., 2002). Biochemical changes associated with seed deterioration might be due to changes in enzyme activity, respiratory and synthetic pathways,

membranes, storage compounds and chromosomes (Ravikumar *et al*., 2002). Specific information on biochemical changes associated with seed viability is required for adopting the best possible storage condition to delay deterioration of seeds.

Seed health is known to have a great impact on determining quality of planting stock. Many microorganisms may get associated with bamboo seeds during pre- and post-harvest period. Seed borne diseases affect quality of the seed, which leads to reduction of seed viability and result in the deterioration of seeds. Improper cleaning and seed storage will be responsible for incidence of storage microorganisms and low seed viability. Specific information on seed borne diseases in most of the commercially important bamboo species is lacking. It is essential for the development of appropriate pest management strategies for increasing productivity of bamboo forests and plantations. In this context, the present study was undertaken to develop appropriate seed handling techniques for commercially important two bamboo species viz., *Dendrocalamus brandisii* and *D. sikkimensis*.

Dendrocalamus brandisii (Munro) Kurz is a large evergreen sympodial edible bamboo, which is commonly used for house construction, making baskets, handicrafts and furniture. It is mostly found in South and North-Eastern India and Myanmar and also introduced to South-East Asia. Natural population in India is limited to tropical forests up to an altitude of 1300 m in Manipur and Andaman. It is widely cultivated in Karnataka and Kerala and the species found growing in the tropical forests, mainly on calcareous rocks up to an altitude of 1300 m (Seethalakshmi & Kumar, 1998). Young shoots are edible and are of good quality (Rao *et al*., 1998). This species is one of the preferred bamboos for cultivation in homesteads due to its thorn-less erect nature. Gregarious flowering is observed for the species with a flowering cycle of 40-45 years. Flowering is reported from Manipur during 1987-88 (Seethalakshmi & Kumar, 1998) and in Coorg areas of Karnataka during 2009-2012.

Dendrocalamus sikkimensis Gamble is also a large bamboo species having caespitose stems with few culms. It is distributed in North-Eastern India, West Bengal, Sikkim, Arunachal Pradesh, Nagaland and Meghalaya (Garo Hills) and can grow up to an altitude of 2100 m (Seethalakshmi & Kumar, 1998). It is used for fencing, posts, huts, ropes, boxes, water pipes, specially for 'Chungas' for carrying water and as animal fodder in the Himalayan region. It is identified as an ideal raw material for pulp and paper (Holstrom, 1993). Gregarious flowering is found with a flowering cycle of 45-60 years. Flowering was reported during 1916, 1932 and 1982 (Seethalakshmi & Kumar, 1998). It flowered gregariously in

Arunachal Pradesh during 2004. The species was introduced in Kerala during 1992 and planted in the Kerala Forest Research Institute campus, Peechi and Bambusetum at Field Research Centre, Velupadam, Thrissur. It flowered in both locations during August, 2009 (Jijeesh *et al*., 2012).

Information on the seed handling techniques of *D. brandisii* and *D. sikkimensis* is lacking. Considering the increasing demand of these two commercially important bamboo species, there is an urgent need to standardize methods for seed collection, processing, storage and handling of seeds to provide high quality seeds for future bamboo planting programmes.

Hence the present study is formulated with the following objectives:

1. To standardize methods for seed collection, processing and testing seeds of different bamboo species.
2. To develop storage methods for orthodox types of bamboo seeds.
3. To assess the factors affecting seed viability such as biochemical changes and infestation with fungi and insects.

2. MATERIALS AND METHODS

The present study was carried out at Kerala Forest Research Institute, Peechi in Thrissur District during 2011-2014.

2.1. Seed collection and processing

Seeds of *Dendrocalamus brandisii* were collected from Ponnampet in Coorg District, Karnataka State (12° 08' 32.5"N; 75°55' 16.0" E) during May, 2011. *D. sikkimensis* seeds were collected from the Field Research Centre, KFRI, located at Velupadam in Thrissur District, Kerala (10° 26' 07.95" N; 76° 21' 32.92" E) during May 2011. Naturally fallen mature seeds were collected on a tarpaulin sheet spread around the clump. Seeds were winnowed, sieved, hand-cleaned of non-seed materials and air-dried.

2.2. Seed characteristics

Seed characteristics such as seed weight, size (length, width & thickness), moisture content (MC %) and physical purity were determined. Moisture content is essential to determine seed viability and storage condition. Seed moisture was measured by oven-dry method (103±2 °C for 17±1 hr) and expressed as percentage and calculated by the following formula (ISTA, 2005):

$$\text{Moisture content (\%)} = \frac{(\text{Fresh weight} - \text{Dry Weight})}{\text{Fresh weight}} \times 100$$

Seed purity was determined as:

$$\text{Purity (\%)} = \frac{\text{Weight of pure seed}}{\text{Total weight of sample}} \times 100$$

2.3. Germination test

Germination tests were carried out under laboratory condition (about 32 °C& 90 % RH) in vermiculite, quartz sand, germination paper and soil. Hundred seeds in four replications were used for the study. Effect of media on seed germination and seedling emergence was studied for 30 days. Vigour index was calculated using the following formula (Abdul-Baki & Anderson, 1973)

Vigour index (VI) = Germination (%) x (Shoot length + Root length or Total seedling length)

2.4. Accelerated ageing test

Seeds were subjected to accelerated ageing at 42±1 ˚C with 100 % relative humidity for 1, 3, 5 and 8 days, in a covered water bath. Germination tests were carried using polyurethane foam sheet as the substratum. The germination medium was maintained moist till the test was over. Germination percentage was calculated using the formula:

$$\text{Germination (\%)} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

2.5. Biochemical assays

Biochemical assays were carried out for protein, starch, total soluble sugars, peroxidase, acid and alkaline phosphatase, and α and β-amylases during first, third, fifth and eighth days of accelerated ageing test. Protein was estimated using the bovine serum albumin as standard.

2.5.1. Protein

Extraction of protein from the sample was carried out using tris-HCl buffer (pH 7.8). The supernatant was measured at 660 nm for protein estimation (Lowry *et al*., 1951).

2.5.2. Starch

Estimation of starch was carried out by treating the sample with 80 % ethanol to remove sugars and then starch was extracted using perchloric acid and anthrone reagent. Glucose was used as standard and absorbance measured at 630 nm (Sadasivam & Manickam, 1996).

2.5.3. Total soluble sugar

For the extraction of total soluble sugars, sample was ground with 10 ml of 80 % methanol and centrifuged at 5000 rpm and the supernatant used for estimation. Concentration of soluble sugars was calculated using glucose as standard and the absorbance read in a UV visible spectrophotometer at 490 nm (Dubois *et al.*, 1956).

2.5.4. Peroxidase

Estimated peroxidase activity by extracting the sample in 3 ml of 0.1 M phosphate buffer (pH 7) by grinding with a pre-cooled mortar and pestle and centrifuged at 18000 rpm at 5 ºC for 15 min. The supernatant was measured for enzyme estimation (Malik & Singh, 1980).

2.5.5. Acid and alkaline phosphatase

Acid and Alkaline phosphatases were estimated using p-nitrophenol as standard. Sample was homogenized in 10 ml of ice-cold buffer solution (Citrate buffer pH 5.3) for acid phosphatase and glycine NaOH buffer (pH 10.4) for alkaline phosphatase in a pre-chilled pestle and mortar. The filtrate obtained was subjected to centrifugation at 10,000 rpm for 10 min. The supernatant was used for enzyme estimation and the absorbance read at 405 nm (Sadasivam & Manickam, 1996).

2.5.6. α and β-amylases

The activities of α and β-amylases were estimated as per the standard method (Sadasivam & Manickam, 1996). Sample was extracted using 0.02 M Sodium phosphate buffer (pH 6.9) for α-amylase and 0.016 M Sodium acetate buffer (pH 4.8) for β-amylase. Starch solution (conc: 1 %) was added to the supernatant and kept at 25 ^{o}C for 3 min. Dinitrosalicylic acid was used as colour reagent and maltose as standard. The absorbance was measured at 540 nm (Bernfeld, 1955).

2.6. Seed storage

Seed longevity was evaluated under different storage conditions at one month interval up to 36 months. Seed samples were divided into 15 lots and 5 lots each was stored at three different storage conditions viz., ambient condition (T_0), 16 ^{o}C (T_1) and 4 ^{o}C (T_2). The samples were stored in sealed double polythene bags having 0.05 mm thickness, kept in air tight plastic containers. Relative humidity (45 %) was maintained in 16 ^{o}C and 4 ^{o}C storage rooms. Seeds from each condition were tested for MC % and viability at monthly interval till 36th month. Germination tests were carried out in all the four media as mentioned earlier. Daily germination counts were recorded for one month.

2.7. Rapid viability tests

Rapid viability tests (TTZ & H_2O_2 test) were carried out to determine the potential germinability of seeds. Hundred seeds in four replications were used for the experiment.

2.7.1. Tetrazolium (TTZ) test

Seeds of *D. brandisii* and *D. sikkimensis* were soaked in water overnight prior to the test. The imbibed seeds were split open and soaked in Tetrazolium solution prepared by dissolving 1 g of TTZ salt (2,3,5-triphenyl tetrazolium chloride) in 1 litre of distilled water. The samples

were incubated in darkness at room temperature for 24 hr. Seeds immersed in distilled water served as control. After the test duration, seeds were thoroughly washed in water and viability was evaluated based on the embryo staining (Moore, 1985).

2.7.2. Hydrogen peroxide (H_2O_2) test

Seeds were soaked in 1% H_2O_2 solution and incubated in darkness at room temperature for 3 days. At the end of third day, H_2O_2 solution was replaced with fresh solution. Seeds were again incubated in darkness for another 3 days. Seeds were evaluated for viability by recording the emergence of radicle (Bhodthipuks *et al*., 1996).

2.8. Seed mycoflora

Seeds from all the storage conditions were subjected for determining fungal incidenceat monthly intervals. Prevalence of different fungi was determined by standard blotter method following the ISTA rules (Anon., 2005). Seeds were placed at an equal distance in sterilized Petri-plates containing moisten three layers of sterilized blotter discs and incubated at 25±2 ^{o}C for 5-7 days. Twenty five seeds were placed in each plate with 4 replications. Fungi were identified on the basis of their morphological characters with the help of standard manuals (Barnett & Hunter, 1972). Percentage frequency of incidence of various fungal species was calculated as follows:

$$\text{Frequency of incidence (\%)} = \frac{\text{No. seeds on which a fungal species occurs}}{\text{Total no. of seeds}} \times 100$$

Effects of biologicals and fungicides on seed mycoflora and seed deterioration were evaluated to develop appropriate storage method.

Following plant species were used to evaluate the effect leaf extract on seed mycoflora:

1. *Azadirachta indica*
2. *Aegle marmelos*
3. *Lawsonia inermis*

Fresh leaves of *A. indica, A. marmelos* and *L. inermis* were used for the study. Leaves were air dried at room temperature. Leaf extracts were prepared by crushing the leaves in a mortar and pestle using distilled water in the ratio 1:1 (50 g leaves crushed in 50 ml water). Seed samples were dipped in the leaf extract for 1 hr. The treated seeds were then air-dried on filter paper. The treated seeds

were tested using standard blotter method (Anon., 2005). Seed samples dipped in distilled water served as control. The fungal occurrence was recorded.

Following fungicides were used to evaluate their effect on seed mycoflora:

1. Bavistin
2. Thiram
3. Indofil M-45

Seeds were mixed with requisite amount of each fungicide and shaken mechanically for 20 min for proper coating. Untreated sample served as control. Determined the effect of fungicides on seed mycoflora by standard blotter method as per ISTA rules (Anon., 2005).

2.9. Statistical analysis

Experimental design used for the study was Completely Randomized Design (CRD). Data were statistically analyzed using 'Analysis of variance' (ANOVA) test.

3. RESULTS AND DISCUSSION

Results of the study on seed handling techniques carried out in *Dendrocalamus brandisii* and *D. sikkimensis* are presented in this section.

3.1. Seed attributes

Seed of *D. brandisii* and *D. sikkimensis* are small, ellipsoidal in shape and deep brown in colour. *D. brandisii* seeds are 3.20 mm long, 2.34 mm wide and 1.97 mm thick. Similarly, *D. sikkimensis* seeds are 3.29 mm long, 2.30 mm wide and 1.86 mm thick. One thousand seed weight of *D. brandisii* was 6.90 g at 8.11 % moisture content and that of *D. sikkimensis* was 5.47 g at 8.33 % moisture content (Table 1).

Table 1. Seed attributes of *Dendrocalamus brandisii* and *D. sikkimensis* (Mean±SE).

Species	1000 seed weight (g)	No. of seeds/kg	Length (mm)	Width (mm)	Thickness (mm)	Moisture content (%)	Physical Purity (%)
D. brandisii	6.90 ± 0.07	1,45,261.37 ± 1578.74	3.20 ± 0.08	2.34 ± 0.07	1.97 ± 0.07	8.11 ± 0.24	95.87 ± 0.22
D. sikkimensis	5.47 ± 0.06	1,83,227.38 ± 2149.85	3.29 ± 0.10	2.30 ± 0.03	1.86 ± 0.04	8.33 ± 0.17	94.65± 0.27

3.2. Effect of germination media on seed germination

Table 2 shows the result of germination trials in different germination media. Higher cumulative germination percentage was recorded in vermiculite medium for *D. brandisii* (60 %) and *D. sikkimensis* (52 %), followed by quartz sand (55 & 48 %), germination paper (55 & 47 %) and soil (36 & 35 %) respectively. Highest seed germination in vermiculite might be due to the greater water retention capacity of vermiculite. Seed germination commenced on the 3rd day after sowing (DAS) and completed in 20 DAS in vermiculite, quartz sand and germination paper. However, germination of seeds in the soil medium started late on 7 DAS and completed in 26 DAS.

Table 2. Seed germination in different media (Mean±SE)

Germination media	Germination %	
	D. brandisii	***D. sikkimensis***
Vermiculite	59.95±0.54	52.00±0.53
Quartz sand	54.55±0.33	48.25±0.64
Germination paper	54.80±0.69	46.95±0.76
Soil	36.15±0.55	35.25±0.31

3.3. Effect of germination media on seedling growth

Performance of seedlings germinated in different media is given in Table 3. Statistical analysis of the result revealed significant differences at 1 % level with respect to shoot length, root length and vigour index. However, there was no significant difference in the number of leaves between the four germination medium.

Table 3. Effect of germination media on seedling growth (Mean±SE).

Germination medium	Species	Shoot length (cm)	Root length (cm)	No. of leaves	Vigour index
Vermiculite	*Db*	10.3±0.168	4.44±0.075	2.38±0.041	885.94±13.511
	Ds	12.25±0.093	5.04±0.05	2.50±0.049	897.71±8.195
Quartz sand	*Db*	8.41±0.152	3.76±0.051	2.33±0.053	664.96±9.253
	Ds	10.53±0.092	4.13±0.068	2.53±0.033	694.97±6.655
Germination paper	*Db*	8.26±0.122	10.32±0.187	2.30±0.056	1025.54±16.739
	Ds	9.24±0.052	11.18±0.079	2.43±0.045	952.32±8.538
Soil	*Db*	8.22±0.069	6.21±0.126	2.33±0.032	516.33±5.587
	Ds	9.16±0.058	6.46±0.106	2.43±0.045	550.23±4.128

Note: Db = Dendrocalamus brandisii; Ds = Dendrocalamus sikkimensis

Generally, the data showed that seedling vigour was more in seeds germinated in vermiculite medium. However, roots of seedlings that germinated in germination paper was lengthiest than that of other medium; hence, the seedling vigour appears as highest. This was due to the hydrotropic movement of roots, i.e., growth of roots in response to the stimulus of water. While using this medium, usually seeds placed on top portion of the germination paper and make it as roll. The roll placed in beaker filled with water. In the case of vermiculite, quartz sand and soil, root growth was normal.

3.4. Accelerated ageing

Accelerated ageing test was carried out to predict the storability of seeds. Germination rate of seeds declined drastically with ageing. An average germination of seeds prior to accelerated ageing was 59.71 % in *D. brandisii* and drastically reduced with ageing. Germination rate decreased to 55.60 % during first day of ageing. Thereafter, it reduced to 44.28, 30.19 and 15.39 % on third, fifth and eighth day of ageing, respectively. With respect to *D. sikkimensis*, seed germination prior to the ageing was 51.49 % and it reduced with ageing. On the first day of ageing germination decreased to 47.05 per cent. Thereafter, it decreased to 35.77, 25.89 and 11.75 per cent respectively on 3rd, 5th and 8th day of ageing. Commencement of seed germination was also different. Seeds kept as control (un-

aged seeds), started germination from the third day of sowing in both the species. As the ageing progressed, the time taken for germination also increased and on the final day of ageing, germination was observed on the tenth day of sowing. Number of seeds germinating on each day was more in control than the aged seeds. Germination of *D. brandisii* seeds decreased from 59.71 to 15.39 % and that of *D. sikkimensis* decreased from 51.49 to 11.75 %. In an earlier study, decreasing trend of germination in seeds of *B. bambos* (73.8 to 51.5 %) and *D. strictus* (67.2 to 38.6 %) with ageing (Ravikumar *et al*., 1998 & 2002) had been reported.

Effect of accelerated ageing on biochemical components

Figures 1-8 depict the biochemical changes during ageing process. Analysis of variance revealed significant difference in biochemical parameters due to ageing at 1 % level. Total soluble proteins, sugars and starch content decreased with ageing. Gradual decrease observed in the activity of acid & alkaline phosphatase and peroxidase. Activity of α-amylase and β-amylase increased during ageing.

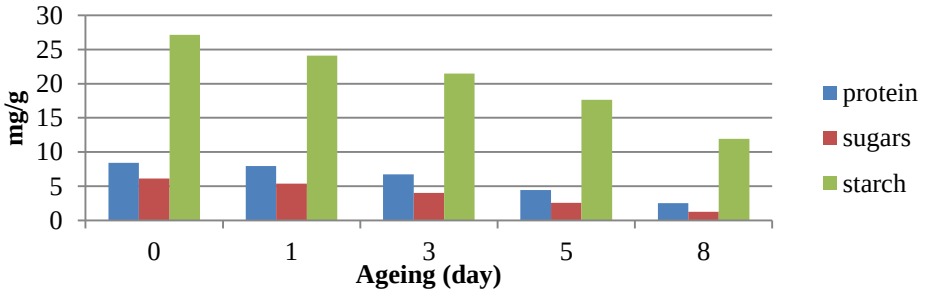

Fig. 1. Variation in protein, sugars and starch content induced by accelerated ageing in *D. brandisii*

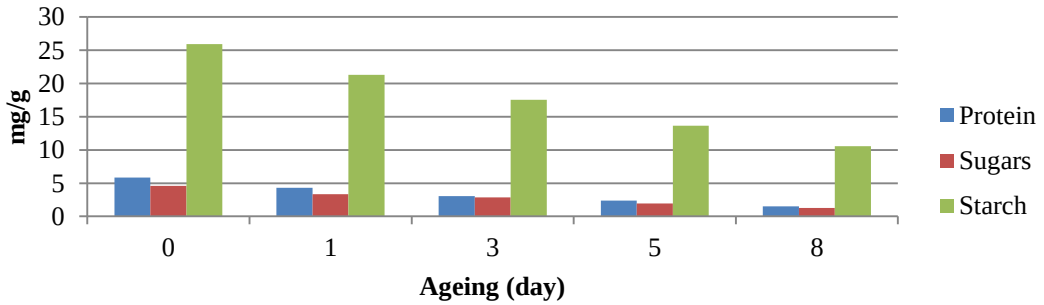

Fig. 2. Variation in protein, sugars and starch content induced by accelerated ageing in *D. sikkimensis*

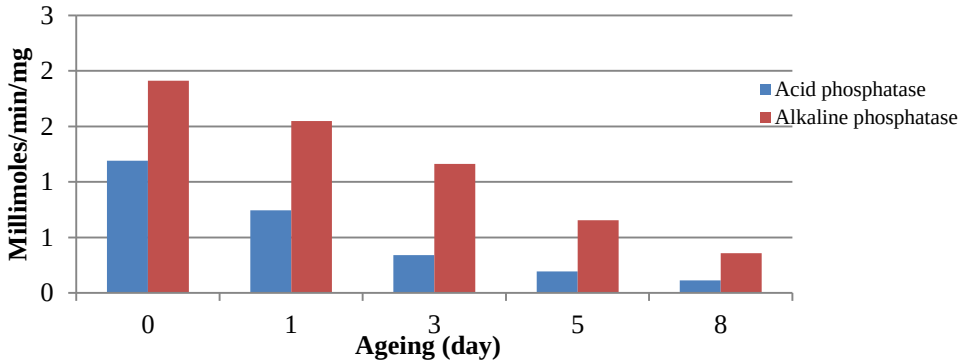

Fig.3.Variation in the activity of acid and alkaline phosphatase induced by ageing in *D. brandisii*

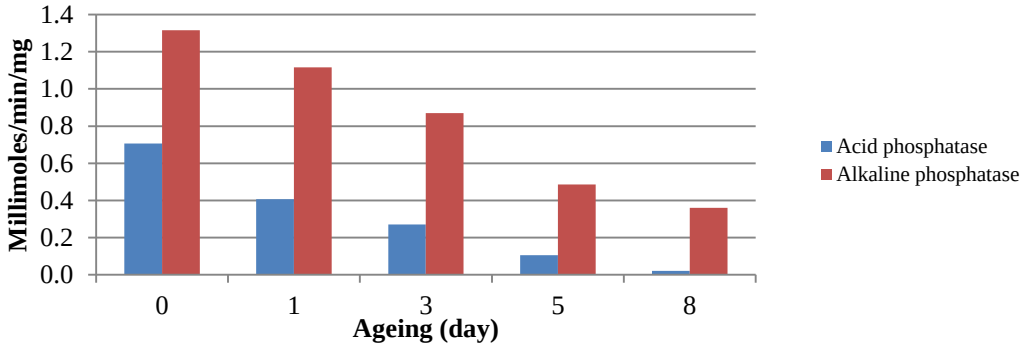

Fig. 4. Variation in the activity of acid and alkaline phosphatase induced by ageing in *D. sikkimensis*

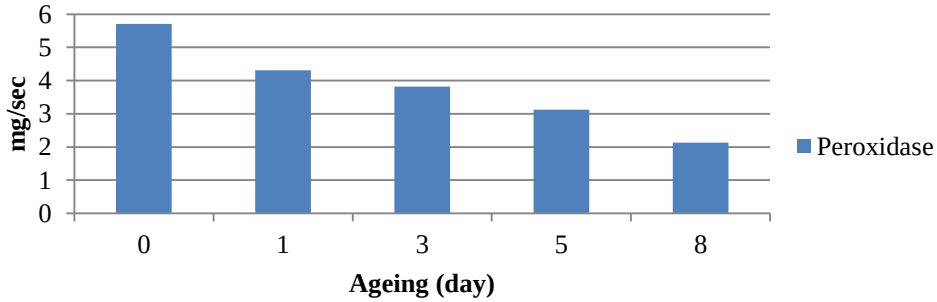

Fig. **5**. Variation in the activity of peroxidase induced by accelerated ageing in *D. brandisii*

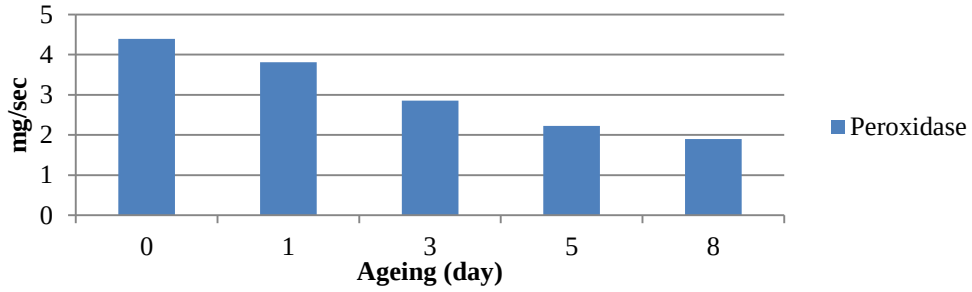

Fig. 6. Variation in the activity of peroxidase induced by accelerated ageing in *D. sikkimensis*

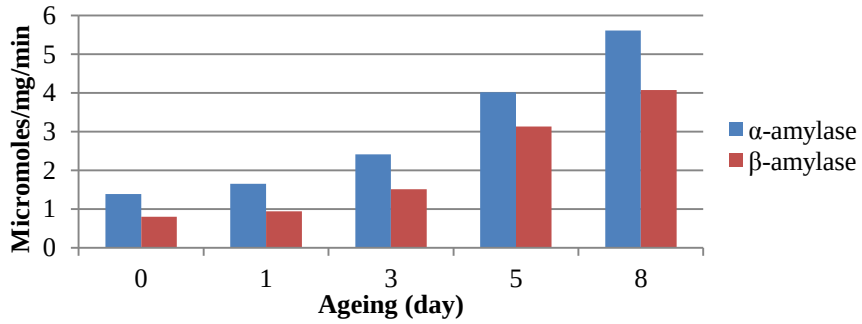

Fig. 7. Variation in the activity of α and β- amylase induced by accelerated ageing in *D. brandisii*

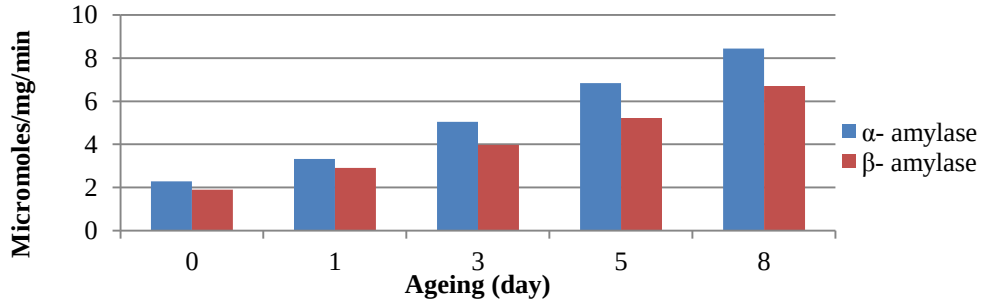

Fig. 8. Variation in the activity of α and β- amylase induced by accelerated ageing in *D. sikkimensis*

Previous studies in *B. bambos* and *D. strictus* reported that the biochemical components such as sugars, proteins and lipids, activity of peroxidase, acid phosphatase and alkaline phosphatase were reduced with accelerated ageing. Increase in total free amino acids and the activity of amylases confirmed the degradation of seed reserves (Ravikumar *et al*., 1998 & 2002). Therefore, degradation of food reserves may be the main reason for seed deterioration, resulting loss of viability. Baldwin (1955) stated that 1 % reduction in moisture content double the storage life of seeds. In this experiment, seeds with lower MC retained their viability for longer duration than that

of higher MC. Therefore seed moisture content, temperature and food reserves influence seed storage and should be the major factors to be considered in the further studies. Variation in the biochemical components in seeds of *D. brandisii* and *D. sikkimensis* were more or less similar to the findings of earlier studies (Ravikumar *et al*., 1998 & 2002; Basavarajappa *et al*., 1991; Bernal-lugo & Leopold, 1992). Increased total free amino acids and the activity of amylases confirmed the degradation of seed reserves as reported by Ravikumar *et al*., (1998 & 2002). The biochemical analysis of *B. bambos* seeds stored at different conditions shows qualitative and quantitative changes in food reserves specially sugars and proteins (Appasamy, 1993). The declined protein content during ageing might be due to degradation by proteinases. Similar results were reported in *Arachis hypogaea* (Rao *et al*., 1970) and *Zea mays* (Basavarajappa *et al*., 1991). During ageing, the starch content in seeds were hydrolyzed by the activity of starch degrading enzymes α and β-amylases, resulting in the decrease of starch content and increase in the activity of amylases. Seed deterioration may be due to the de-naturation of biomolecules, accumulation of toxic substances and loss of membrane integrity (Roberts, 1972; Basavarajappa *et al*., 1991). The decreased activity of phosphatases and peroxidases during ageing may adversely affect the metabolism of seeds which results in the deterioration of seeds and loss of viability.

3.5. Seed storage

The results presented in figures 9 and 10 clearly show that the best seed storage condition for *D. brandisii* and *D. sikkimensis* was 4 ^{o}C and 45 % RH. The study indicated that seeds of both the species can be stored at 4 ^{o}C with 45 % RH for 36 months without much reduction of viability. Average seed germination at 36 month storage was 60.65 and 52.10 % respectively for *D. brandisii* and *D. sikkimensis*. Seeds stored at 16 ^{o}C were able to retain viability up to 10 months in both the species (54.15 & 46.70 % respectively). Gradually, it decreases from 11th month onwards and totally lost at the end of 24th month. However, at ambient temperature, seed viability was stable only for five months in *D. brandisii* (52.70 %) and four months in *D. sikkimensis* (52.25 %), there zafter decreased and lost completely from 11 month onwards. Statistical analysis (ANOVA) revealed that storage temperature had significantly influenced on seed longevity of both the species (Table 6). As seeds of both the species exhibit orthodox seed physiology, they could be stored at RH less than 45 per cent; but it will be expensive to maintain a lower RH.

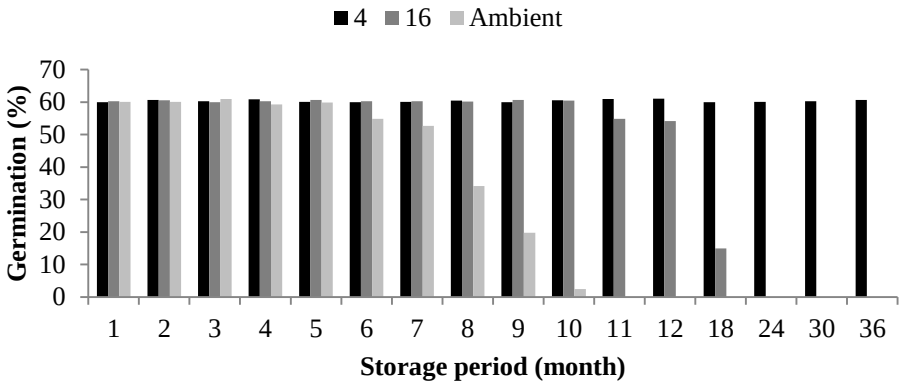

Fig. 9. Germination pattern of *D. brandisii* seeds under storage

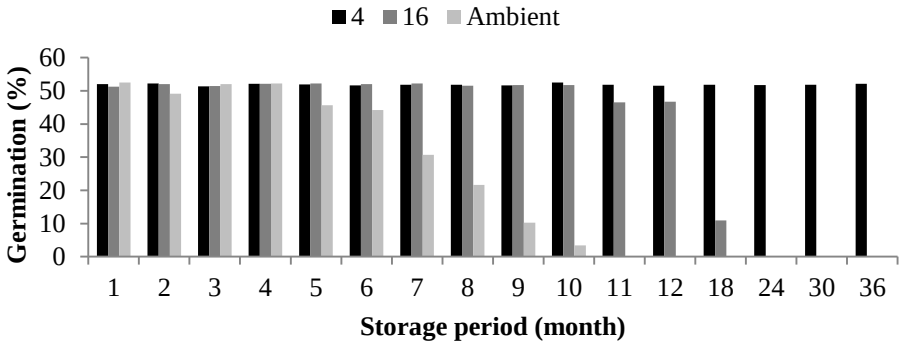

Fig. 10. Germination pattern of *D. sikkimensis* seeds under storage

Note (Figs. 1 & 2): 4 = 4 ºC & 45 % RH; 16 = 16 ºC & 45 % RH; ambient = Room temperature (about 32 ºC)

3.6. Effect of storage temperature on seed viability

Seed viability of both the species was significantly influenced by storage temperature (Figs. 9 & 10; Table 6). Seeds of *D. brandisii* and *D. sikkimensis* were able to maintain their viability (60.65 & 52.10 %) up to 36 months in cold condition (4 ºC & 45 % RH). The result is substantiated with finding of Boonarutee and Somboon (1990) that seed viability can be extended for 18 months by storing under cold condition (2-4 ºC). Earlier studies on *Bambusa arundinacea, B. nutans, D. strictus, D. membranaceus* and *Thyrsostachys siamensis* showed more or less similar results (Gupta & Sood, 1978; Somen & Seethalakshmi, 1989; Thapliyal *et al*., 1991; Rawat *et al*, 2003; Warrier *et al*., 2004).

3.7. Effect of storage temperature on seed moisture content

Seed viability of *D. brandisii* and *D. sikkimensis* could be extended by reducing MC % up to a critical level (8 %) prior to the storage. Maintaining the critical level of seed moisture was

influenced by storage temperature. Seeds of both the species were able to maintain their lowest safe moisture level up to 36 months in cold storage (4 ºC & 45 % RH) condition(Figs. 11 & 12).

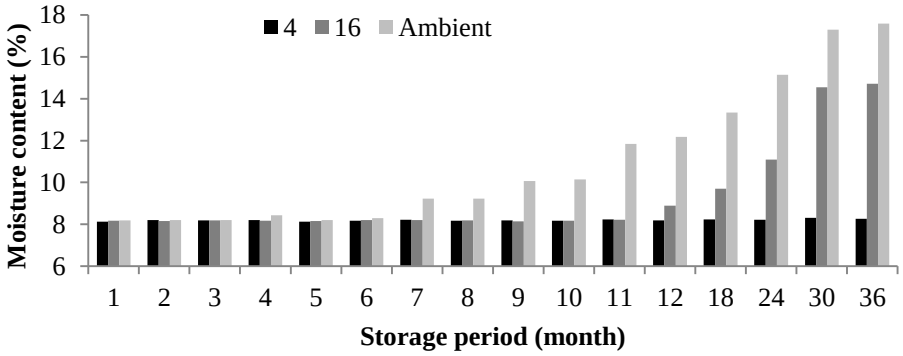

Fig. 11. Moisture content of *D. brandisii* seeds under storage

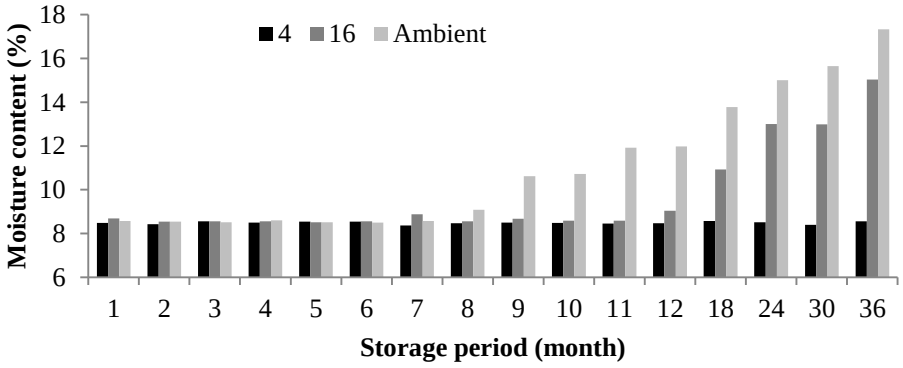

Fig. 12. Moisture content of *D. sikkimensis* seeds under storage

Note (Figs. 3 & 4): 4 = 4 ºC & 45 % RH; 16 = 16 ºC& 45 % RH; ambient = Room temperature (32 ºC)

3.8. Seed viability test (Rapid Viability test using TTZ & H_2O_2 vs. Germination)

Results of various viability tests given in Table 4 and 5 did not show any significant difference between TTZ (2,3,5-triphenyl tetrazolium chloride), Hydrogen peroxide (H_2O_2) and germination tests. In TTZ test, the embryo stained with pink colour was considered as viable seed. Whereas in H_2O_2 test, seed is considered viable when the radicle length exceeds 3 mm length after 7 days of incubation (Bhodthipuks *et al*., 1996). ANOVA revealed that no significant difference in results between TTZ, H_2O_2 and germination tests irrespective of storage temperature (Table 6). Hence, rapid viability tests shall be used for immediate assessment of seed viability.

Table 4. Comparison of rapid viability tests and germination test on *D. Brandisii* seeds under storage (Mean±SD)

Storage period (Month)	4 ºC			16 ºC			Ambient		
	TTZ	H_2O_2	Germ	TTZ	H_2O_2	Germ	TTZ	H_2O_2	Germ
1	60.15 ±1.53	61.20 ±1.54	59.95 ±0.54	60.90 ±1.02	59.70 ±1.59	60.25 ±0.47	60.25 ±0.91	60.60 ±1.60	60.05 ±1.19
2	60.60 ±1.82	60.70 ±1.59	60.60 ±0.70	59.80 ±1.28	60.65 ±1.79	60.50 ±0.31	59.75 ±0.97	59.60 ±1.05	60.05 ±0.57
3	60.00 ±1.49	61.55 ±1.54	60.25 ±0.35	60.40 ±1.14	59.85 ±1.63	59.95 ±0.27	59.65 ±1.18	59.85 ±1.50	60.90 ±0.38
4	60.70 ±1.22	60.65 ±1.63	60.85 ±0.82	59.15 ±1.53	59.95 ±1.54	60.25 ±0.18	59.85 ±0.99	59.75 ±1.12	59.25 ±0.18
5	59.90 ±1.41	60.30 ±1.81	60.10 ±0.38	59.80 ±1.58	60.40 ±1.76	60.60 ±0.52	59.85 ±1.42	59.95 ±1.39	59.85 ±0.29
6	60.30 ±1.22	60.45 ±1.50	60.00 ±0.25	59.40 ±1.54	60.10 ±1.62	60.25 ±0.73	56.20 ±1.61	56.35 ±2.72	54.85 ±0.58
7	60.15 ±1.50	60.15 ±1.39	60.10 ±0.29	59.70 ±1.53	60.20 ±1.58	60.30 ±0.48	53.45 ±2.01	53.40 ±2.58	52.70 ±0.48
8	60.15 ±1.27	60.40 ±1.27	60.45 ±0.48	59.25 ±1.65	60.05 ±1.64	60.15 ±0.38	35.00 ±1.12	36.80 ±2.07	34.10 ±0.49
9	59.60 ±1.35	60.25 ±1.45	60.00 ±0.43	59.05 ±1.57	59.50 ±1.28	60.65 ±0.88	20.25 ±1.80	18.80 ±1.44	19.80 ±0.65
10	59.90 ±1.62	60.30 ±1.34	60.55 ±0.41	59.40 ±1.60	59.75 ±1.94	60.40 ±0.68	3.10 ±0.97	3.45 ±1.00	2.45 ±0.21
11	59.65 ±1.18	59.65 ±1.18	60.90 ±0.42	55.55 ±1.88	56.45 ±1.76	54.85 ±0.76	0.00	0.00	0.00
12	59.85 ±0.99	60.25 ±1.16	61.00 ±0.73	53.60 ±1.76	52.95 ±1.50	54.15 ±0.38	0.00	0.00	0.00
18	60.05 ±1.00	60.25 ±1.29	60.00 ±0.61	14.85 ±1.39	14.95 ±1.47	14.90 ±0.65	0.00	0.00	0.00
24	59.85 ±1.50	60.55 ±2.06	60.05 ±0.45	0.00	0.00	0.00	0.00	0.00	0.00
30	60.05 ±1.47	59.75 ±1.25	60.30 ±0.65	0.00	0.00	0.00	0.00	0.00	0.00
36	59.90 ±1.55	59.70 ±1.30	60.65 ±0.58	0.00	0.00	0.00	0.00	0.00	0.00

Note: TTZ = Tetrazolium test; H_2O_2 = Hydrogen peroxide test; Germ = Germination test

Table 5. Comparison of rapid viability tests and germination test on *D. Sikkimensis* seeds under storage (Mean±SD)

Storage period (month)	4 ºC			16 ºC			Ambient		
	TTZ	H_2O_2	Germ	TTZ	H_2O_2	Germ	TTZ	H_2O_2	Germ
1	52.25 ±1.74	52.00 ±1.74	52.00 ±0.53	51.95 ±1.87	52.30 ±1.78	51.30 ±1.47	52.35 ±1.42	51.95 ±1.31	52.55 ±0.82
2	53.30 ±1.55	52.85 ±1.59	52.25 ±0.53	52.25 ±1.61	51.25 ±1.80	52.05 ±0.62	52.00 ±1.52	51.00 ±1.74	49.15 ±1.22
3	51.25 ±1.77	52.10 ±1.61	51.40 ±0.45	51.60 ±1.46	51.10 ±1.77	51.45 ±0.45	51.45 ±1.19	50.65 ±1.49	52.05 ±0.37
4	50.95 ±1.35	51.30 ±1.45	52.15 ±0.42	52.30 ±1.17	51.55 ±1.31	52.15 ±0.45	51.95 ±0.94	51.25 ±1.77	52.25 ±0.31
5	52.40 ±0.88	51.85 ±1.42	51.95 ±0.54	51.65 ±1.08	51.45 ±1.35	52.20 ±0.33	47.00 ±2.00	47.35 ±2.58	45.65 ±0.42
6	51.80 ±1.32	51.65 ±1.53	51.65 ±0.58	52.10 ±1.41	51.30 ±1.52	52.00 ±0.59	43.40 ±1.98	45.65 ±2.73	44.25 ±0.31
7	51.85 ±1.95	52.05 ±1.66	51.80 ±0.69	51.95 ±1.23	51.30 ±1.41	52.20 ±0.33	32.90 ±1.99	32.55 ±2.32	30.75 ±0.50
8	50.65 ±1.34	51.85 ±1.72	51.85 ±0.74	51.60 ±1.14	51.45 ±1.84	51.55 ±0.33	24.05 ±1.31	24.90 ±1.86	21.65 ±0.89
9	51.55 ±1.53	51.50 ±1.39	51.65 ±0.45	51.45 ±1.46	50.30 ±1.17	51.70 ±0.27	9.65 ±1.22	9.90 ±1.29	10.30 ±0.51
10	51.35 ±1.53	52.10 ±1.48	52.55 ±0.41	50.75 ±2.04	51.05 ±2.01	51.70 ±0.54	3.15 ±1.22	2.70 ±0.92	3.45 ±0.48
11	51.30 ±1.80	52.20 ±1.67	51.85 ±0.28	46.45 ±1.82	47.65 ±1.49	46.50 ±0.39	0.00	0.00	0.00
12	52.20 ±1.90	51.90 ±1.68	51.55 ±0.33	45.55 ±1.31	45.35 ±1.56	46.70 ±0.48	0.00	0.00	0.00
18	51.50 ±1.53	51.80 ±1.32	51.80 ±0.41	10.25 ±1.29	10.25 ±1.25	10.90 ±0.38	0.00	0.00	0.00
24	51.20 ±1.15	51.95 ±1.82	51.70 ±0.57	0.00	0.00	0.00	0.00	0.00	0.00
30	51.40 ±1.81	51.05 ±1.50	51.80 ±0.21	0.00	0.00	0.00	0.00	0.00	0.00
36	51.55 ±1.50	51.35 ±1.56	52.10 ±0.55	0.00	0.00	0.00	0.00	0.00	0.00

Note: TTZ = Tetrazolium test; H_2O_2 = Hydrogen peroxide test; Germ = Germination test

Table 6. ANOVA Table: significance levels of seed viability under different storage temperature and comparison between various viability tests

Source	Degrees of freedom (d.f)	Sum of squares (S.S)	Mean sum of squares (M.S)	F - ratio	F – value at 0.05
Storage temperatures (4 °C, 16 °C & ambient)	2	23221.07	11610.54	24.45174*	3
Viability tests (TTZ, H_2O_2 & Germination)	2	1.187	0.5939	0.00125ns	3
Interactions	4	1.87	0.4687	0.00098 ns	2.57
Residual	135	64102.67	474.834		
Total	143	87326.81			

*Note: ns = non-significant; * = significant at P ≤ 0.05*

3.9. Seed mycoflora

Frequency of occurrence of seed mycoflora

Frequency of fungal incidence associated with seeds stored under 4 °C, 16 °C and ambient temperature in different storage period is depicted in figures 13 to 18. A total of 11 fungal species were identified on the seed samples of *D. brandisii*, which include *Alternaria tenuissima, Aspergillus flavus, A. niger, A. versicolor, Chaetomium spirulina, Cladosporium hirtum, Curvularia lunata, Fusarium oxysporum, Penicillium chrysogenum, Rhizopus stolonifer and Stysanus* sp. Similarly, 13 species were identified on the seeds of *D. sikkimensis* and 11 of them were the same species as noticed on *D. brandisii*. Two species viz., *Aspergillus fumigates* and *Penicillium sclerotiorum* exclusively found on the seeds of *D. sikkimensis*. However, *A. tenuissima* was reported only during the earlier period of storage. Occurrence of mycoflora was minimal on seeds stored under 4 °C with 45 % RH.

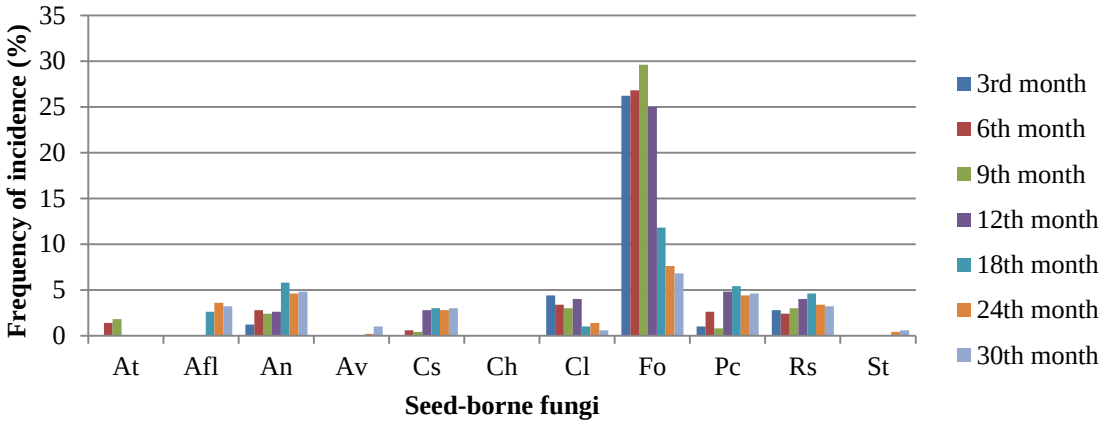

Fig. 13. Fungi associated with *D. brandisii* seeds stored in 4 ºC

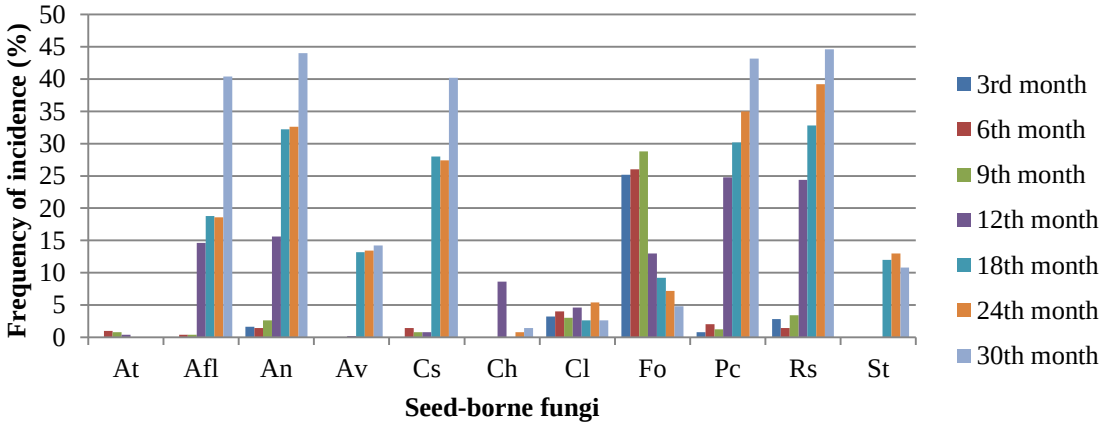

Fig. 14. Fungi associated with *D. brandisii* seeds stored in 16 ºC

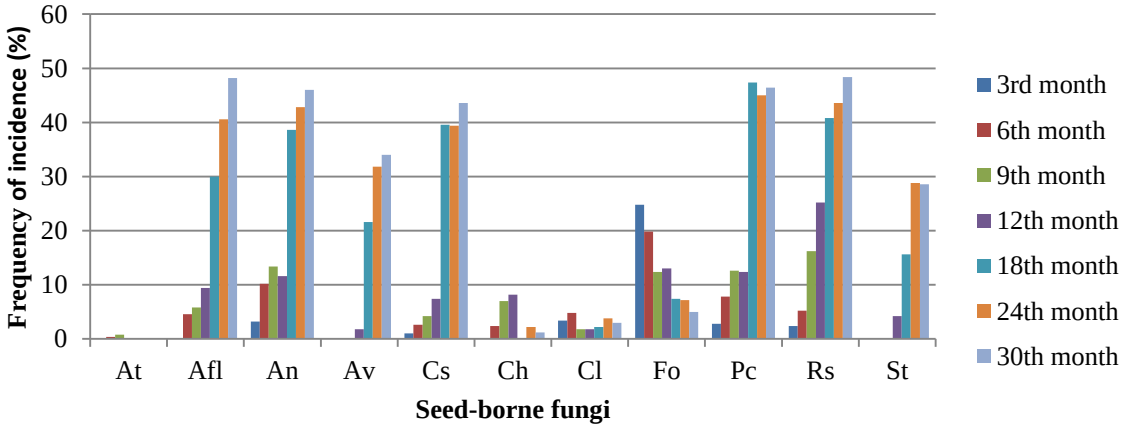

Fig. 15. Fungi associated with *D. brandisii* seeds stored in ambient temperature

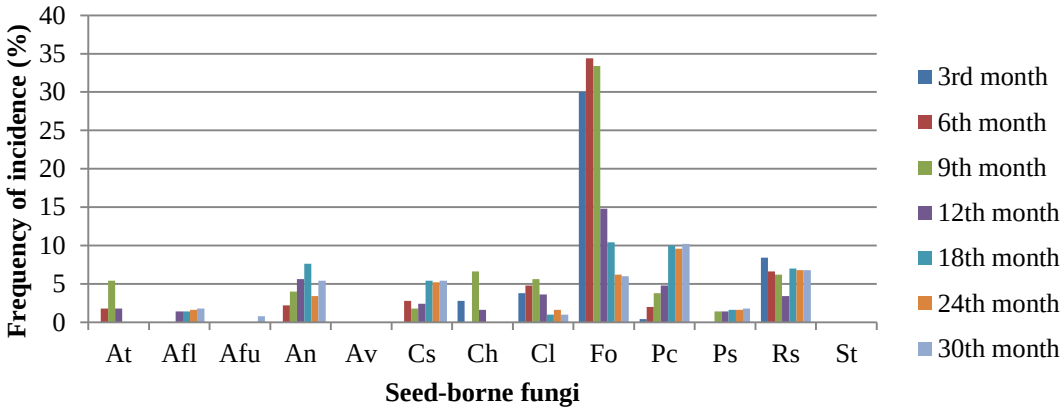

Fig. 16. Fungi associated with *D. sikkimensis* seeds stored at 4 ºC

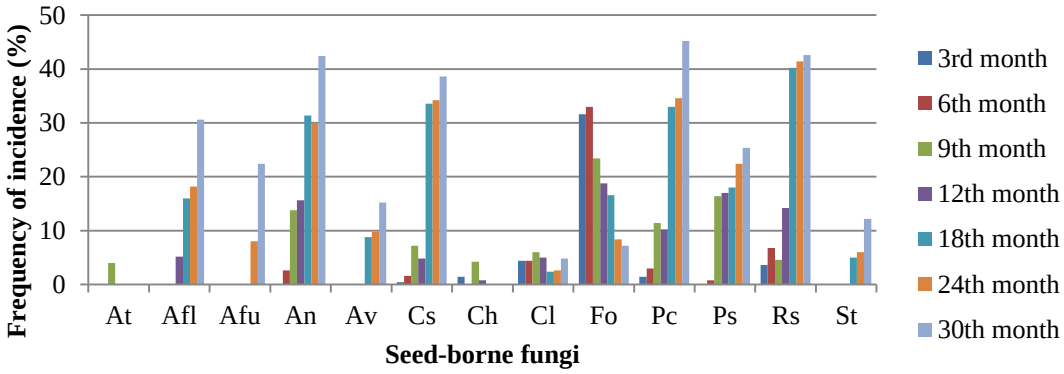

Fig. 17. Fungi associated with *D. sikkimensis* seeds stored at 16 ºC

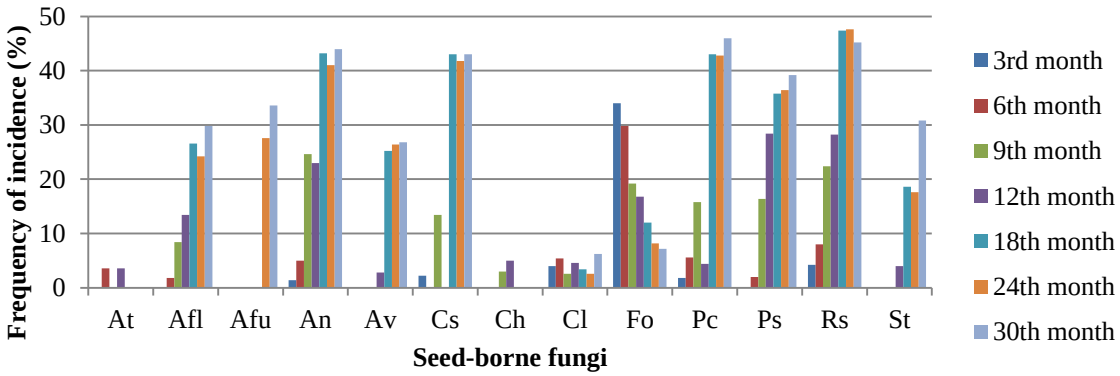

Fig. 18. Fungi associated with *D. sikkimensis* seeds stored at ambient temperature

Note (Figs. 15-20): At = *Alternaria tenuissima;* Afl = *Aspergillus flavus;* Afu = *Aspergillus fumigates;* An = *Aspergillus niger;* Av = *Aspergillus versicolor;* Cs = *Chaetomium spirulina;* Ch= *Cladosporium hirtum;* Cl= *Curvularia lunata;* Fo = *Fusarium oxysporum;* Pc= *Pencillium chrysogenum;* Ps = *P. sclerotiorum;* Rs = *Rhizopus stolonifer; St = Stysanus sp.*

3.10. Effect of biologicals and fungicides on seed-borne mycoflora

Effect of leaf extract of *Azadirachta indica, Aegle marmelos* and *Lawsonia inermis* and chemical fungicides such as bavistin, thiram and indofil on seed mycoflora was evaluated. The treatments reduced seed borne mycoflora and seed deterioration. Among the treatment with biologicals, leaf extract of *A. indica* exhibits significant effect. With respect to fungicides, indofil followed by thiram significantly reduced mycoflora and seed deterioration (Table 7 & 8).

The study indicated that reduction of seed germination during storage might also be due to the activities of fungi resulting seed deterioration and viability loss, this finding is agreement with earlier report by Ora *et al*. (2011). Another study on cultivated hybrid rice varieties revealed that infection of seed-borne pathogen is more in seeds having poor germination. Seed-borne pathogens are responsible for reducing seed quality, protein and carbohydrate contents, reduction in germination capability as well as seedling damage, which results in the reduction of crop yield. Majority the fungi identified are known to produce mycotoxins, which are harmful for human health. *Aspergillus* species are common fungal contaminants of cereals and produce mycotoxins (Bakan *et al.,* 2002; Toth & Teren, 2005). Use of biologicals such as Neem extract, and fungicides such as indofil and thiram for treating seeds, can effectively reduce mycoflora and help to reduce seed deterioration. Proper seed storage of *D. brandisii* and *D. sikkimensis* under low temperature and optimum moisture content is necessary to prevent fungal incidence, mycotoxin production and seed deterioration.

Table 7. Frequency of fungal occurrence during 30th month of storage in *D. brandisii* seeds treated with fungicides and biologicals

Fungus	Storage temperature	Treatment						
		Control	Biologicals (leaf extract)			Fungicide		
			A. indica	*A. marmelos*	*L. inermis*	Bavistin	Thiram	Indofil
Aspergillus flavus	4	3.2	0.0	0.8	1.6	2.8	0.0	0.0
	16	40.4	0.0	7.6	11.4	38.8	0.0	0.0
	ambient	48.2	0.0	11.6	11.4	47.4	0.0	0.0
Aspergillus niger	4	4.8	0.4	1.2	2.4	4.8	0.2	0.0
	16	44.0	0.8	13.8	15.0	43.8	1.2	0.0
	ambient	46.0	1.0	14.8	15.4	46.2	1.6	0.0
Aspergillus versicolor	4	1.0	0.0	0.0	0.0	0.0	0.0	0.0
	16	14.2	0.0	0.0	0.8	13.0	0.0	0.0
	ambient	34.0	0.0	0.0	1.0	32.6	0.0	0.0
Chaetomium spirulina	4	3.0	0.0	0.2	0.4	1.0	0.0	0.0
	16	40.2	0.0	8.6	2.2	34.0	0.0	0.0
	ambient	43.6	0.0	10.2	5.4	37.0	0.0	0.0
Cladosporium hirtum	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	16	1.4	0.0	0.0	0.0	1.4	0.0	0.0
	ambient	1.2	0.0	0.0	0.0	1.4	0.0	0.0
Curvularia lunata	4	0.6	0.0	0.0	0.2	0.8	0.0	0.0
	16	2.6	0.0	0.4	0.6	2.4	0.0	0.0
	ambient	3.0	0.0	1.2	0.6	2.6	0.0	0.0
Fusarium oxysporum	4	6.8	0.8	0.8	1.2	3.2	1.0	0.4
	16	4.8	0.2	0.6	0.8	2.8	0.8	0.2
	ambient	5.0	0.0	0.6	1.0	2.8	0.6	0.4
Pencillium chrysogenum	4	4.6	0.0	0.6	1.4	4.4	0.2	0.2
	16	43.2	0.0	4.4	4.0	42.6	1.4	0.6
	ambient	46.4	0.0	9.2	6.6	46.2	1.2	0.6
Rhizopus stolonifer	4	3.2	0.0	0.0	0.0	3.4	0.0	0.0
	16	44.6	0.0	0.0	0.0	44.2	0.0	0.0
	ambient	48.4	0.0	0.0	0.0	48.6	0.0	0.0
Stysanus sp.	4	0.6	0.0	0.0	0.0	0.2	0.0	0.0
	16	10.8	0.0	0.2	0.6	10.0	0.0	0.0
	ambient	28.6	0.0	0.8	1.0	27.8	0.0	0.0

Table 8. Frequency of fungal occurrence during 30th month of storage in *D. sikkimensis* seeds treated with fungicides and biologicals

Fungi	Storage temperature	Treatment						
		Control	Biologicals (leaf extract)			Fungicide		
			A. indica	*A. marmelos*	*L. inermis*	Bavistin	Thiram	Indofil
Aspergillus flavus	4	1.8	0.0	0.4	1.0	1.2	0.0	0.0
	16	30.6	0.0	4.4	12.2	27.4	0.0	0.0
	ambient	29.8	0.0	7.8	12.8	27.2	0.0	0.0
Aspergillus fumigates	4	0.8	0.0	0.0	0.0	0.6	0.0	0.0
	16	22.4	0.4	1.6	0.0	22.0	0.0	0.0
	ambient	33.6	0.4	1.4	0.0	33.4	0.0	0.0
Aspergillus niger	4	5.4	0.0	0.4	0.6	5.2	0.0	0.0
	16	42.4	0.8	10.2	13.4	40.0	1.0	0.0
	ambient	44.0	0.8	10.6	13.2	43.0	1.4	0.0
Aspergillus versicolor	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	16	15.2	0.0	0.0	0.4	13.0	0.0	0.0
	ambient	26.8	0.0	0.0	1.0	24.8	0.0	0.0
Chaetomium spirulina	4	5.4	0.0	1.0	0.4	3.8	0.0	0.0
	16	38.6	0.0	15.4	3.8	30.2	0.0	0.0
	ambient	43.0	0.0	21.8	5.2	34.6	0.0	0.0
Cladosporium hirtum	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	16	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	ambient	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Curvularia lunata	4	1.0	0.0	0.0	0.0	0.6	0.0	0.0
	16	4.8	0.0	0.6	0.8	2.8	0.0	0.0
	ambient	6.2	0.0	1.0	1.2	5.0	0.0	0.0
Fusarium oxysporum	4	6.0	0.4	0.4	0.8	4.4	0.4	0.0
	16	7.2	0.8	1.0	1.2	5.8	1.0	0.4
	ambient	7.2	0.8	1.0	1.0	5.4	0.8	0.6
Pencillium chrysogenum	4	10.2	0.0	0.8	1.4	9.6	0.4	0.0
	16	45.2	0.0	3.4	5.0	42.2	1.4	0.6
	ambient	46.0	0.0	7.8	9.8	44.2	1.2	0.8
P. sclerotiorum	4	1.8	0.0	0.0	0.0	1.6	0.0	0.0
	16	25.4	0.0	0.2	0.4	22.6	0.0	0.0
	ambient	39.2	0.0	1.0	1.4	36.2	0.0	0.0
Rhizopus stolonifer	4	6.8	0.0	0.0	0.0	6.6	0.0	0.0
	16	42.6	0.0	0.0	0.0	42.0	0.0	0.0
	ambient	45.2	0.0	0.0	0.0	43.6	0.0	0.0
Stysanus sp.	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	16	12.2	0.0	0.4	0.8	11.6	0.0	0.0
	ambient	30.8	0.0	1.0	1.2	26.6	0.0	0.0

4. CONCLUSIONS

The study concluded that seed weight of *D. brandisii* was about 1,45,261 seeds per kg at 8.11 per cent moisture content (MC) and 1,83,227 seeds per kg at 8.33 per cent MC for *D. sikkimensis*. Vermiculite was the best germination medium for both the species, where germination commenced on 3rd day after sowing and completed in 20 days with 60 and 52 per cent cumulative germination. Accelerated ageing shall be used as an ideal test for predicting relative storability of seeds. During accelerated ageing process, germination of seeds decreased from 59.71 to 15.39 per cent in *D. brandisii* and 51.49 to 11.75 per cent in *D. sikkimensis*. Changes in biochemical components like total soluble proteins, sugars and starch content, activity of enzymes like acid and alkaline phosphatase, peroxidase, α-amylase and β-amylases are directly related to seed viability. Total soluble proteins, sugars and starch content decreased with ageing. Similarly, the activity of phosphatase and peroxidase decreased and α-amylase and β-amylase increased during ageing resulting deterioration of seeds and loss of viability. Seeds with lower MC (8 %) retained their viability for longer duration. Storage temperature and relative humidity (RH) had significant influence on seed longevity of both the species. The best storage condition was 4 ^{o}C and 45 % RH, where seeds of both the species were able to maintain viability and lowest safe moisture level for 36 months. Rapid viability tests (TTZ & H_2O_2) did not show any significant difference with germination test; hence, shall be used for immediate assessment of seed viability. Seed mycoflora is one of the major factors affecting seed viability. Eleven fungal species were identified on stored seeds of *D. brandisii* and 13 species in *D. sikkimensis*. Occurrence of mycoflora was minimal on seeds stored at 4 ^{o}C. Treatment with biologicals like extract of Neem (*Azadirachta indica*) leaves and fungicides like indofil and thiram effectively reduced incidence of mycoflora and seed deterioration. Proper seed storage is necessary to prevent fungal incidence, mycotoxin production and seed deterioration. Hence, the present study suggests that storage of bamboo seeds treated with extract of Neem leaves under low temperature and lowest safe moisture level shall reduce seed deterioration and prolong seed longevity.

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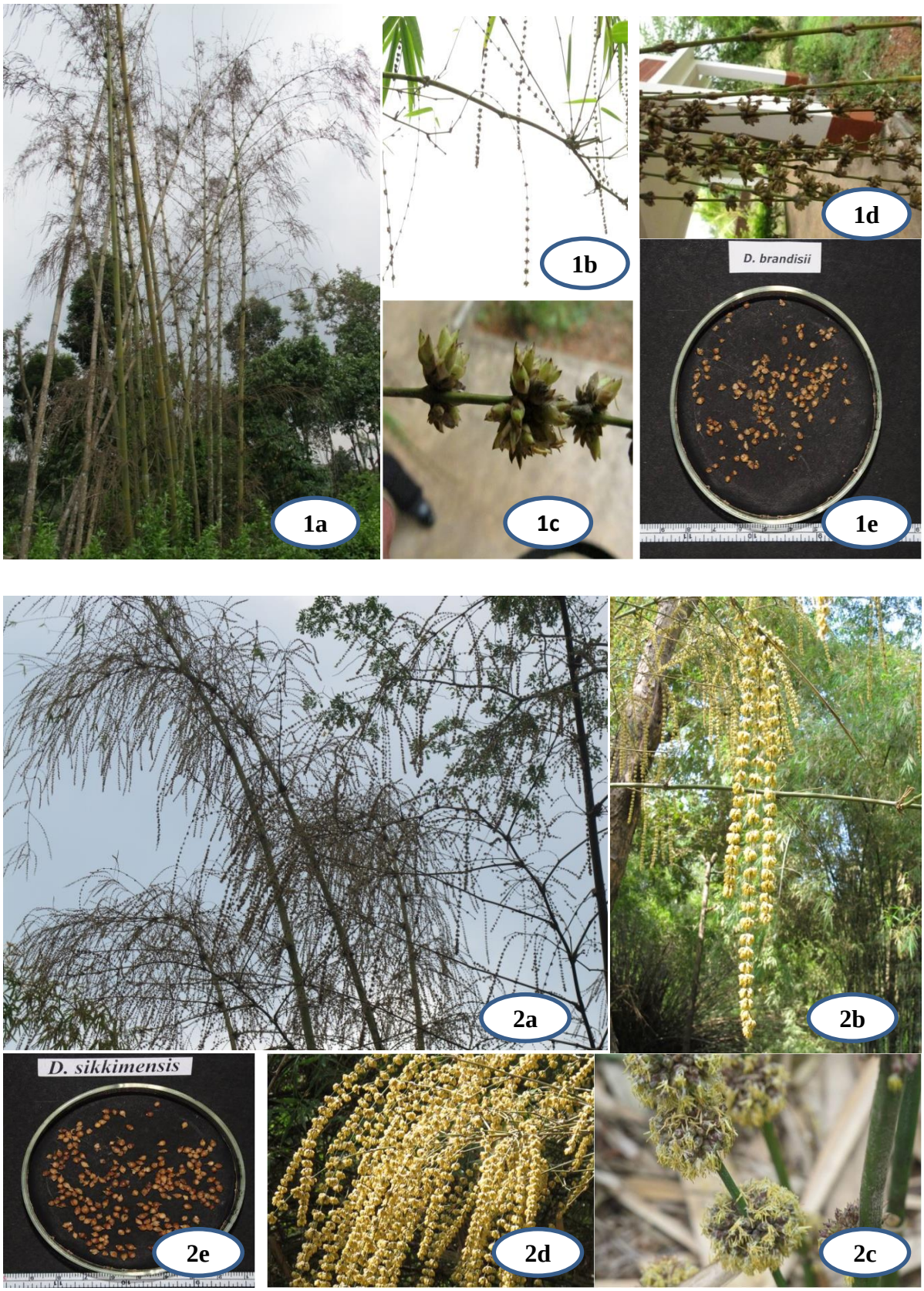

Plate 1. (1) *Dendrocalamus brandisii*: 1a. Flowering clump; 1b, 1c & 1d. Inflorescence; 1e. Seeds.
(2) *D. sikkimensis*: 2a. Flowering clump; 2b, 2c & 2d. Inflorescence; 2e. Seeds

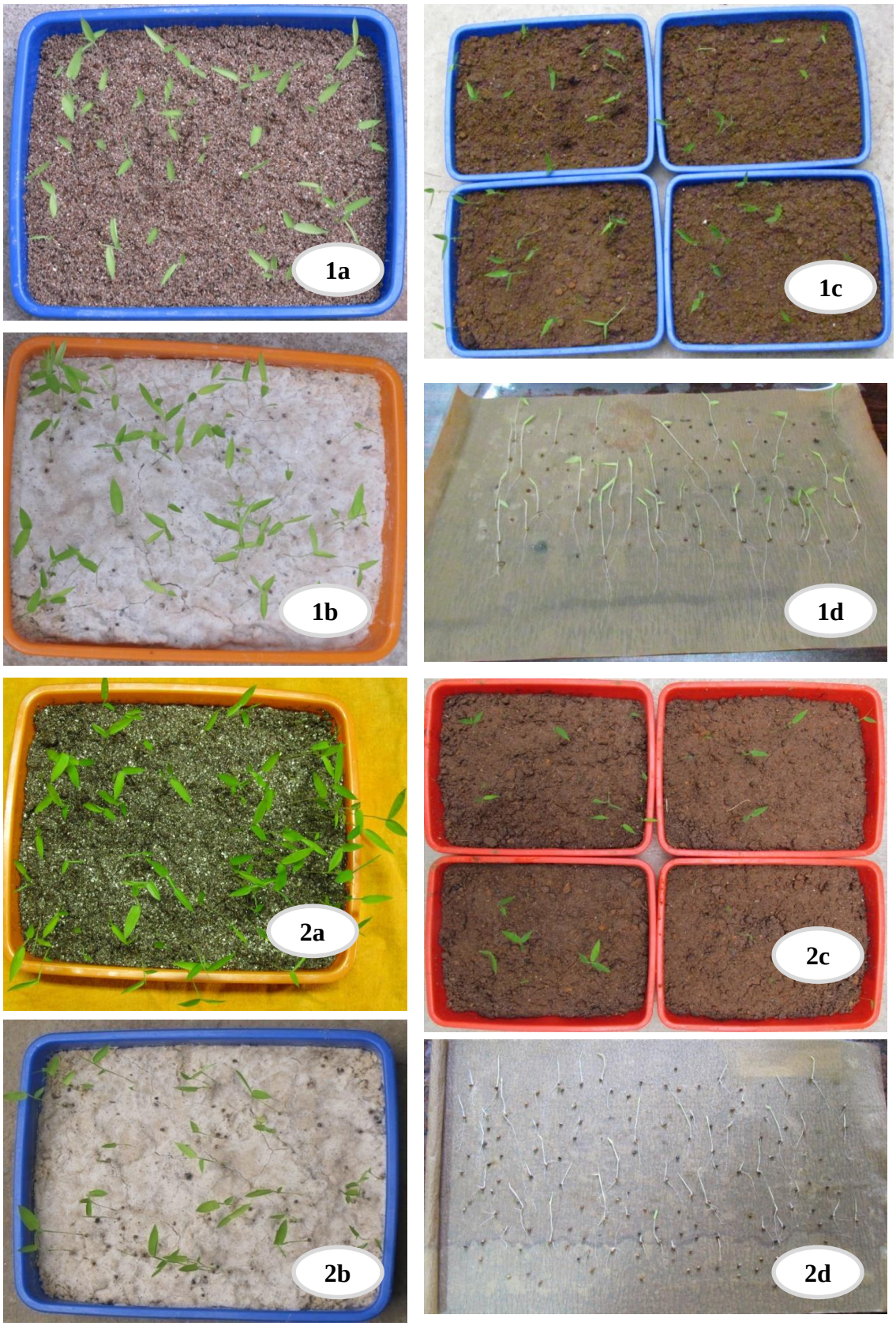

Plate 2. Seed germination in various media:

(1) *D. brandisii*: 1a. Vermiculite; 1b. Quartz sand; 1c. Soil; 1d. Germination paper

(2) *D. sikkimensis*: 2a. Vermiculite; 2b. Quartz sand; 2c. Soil; 2d. Germination paper

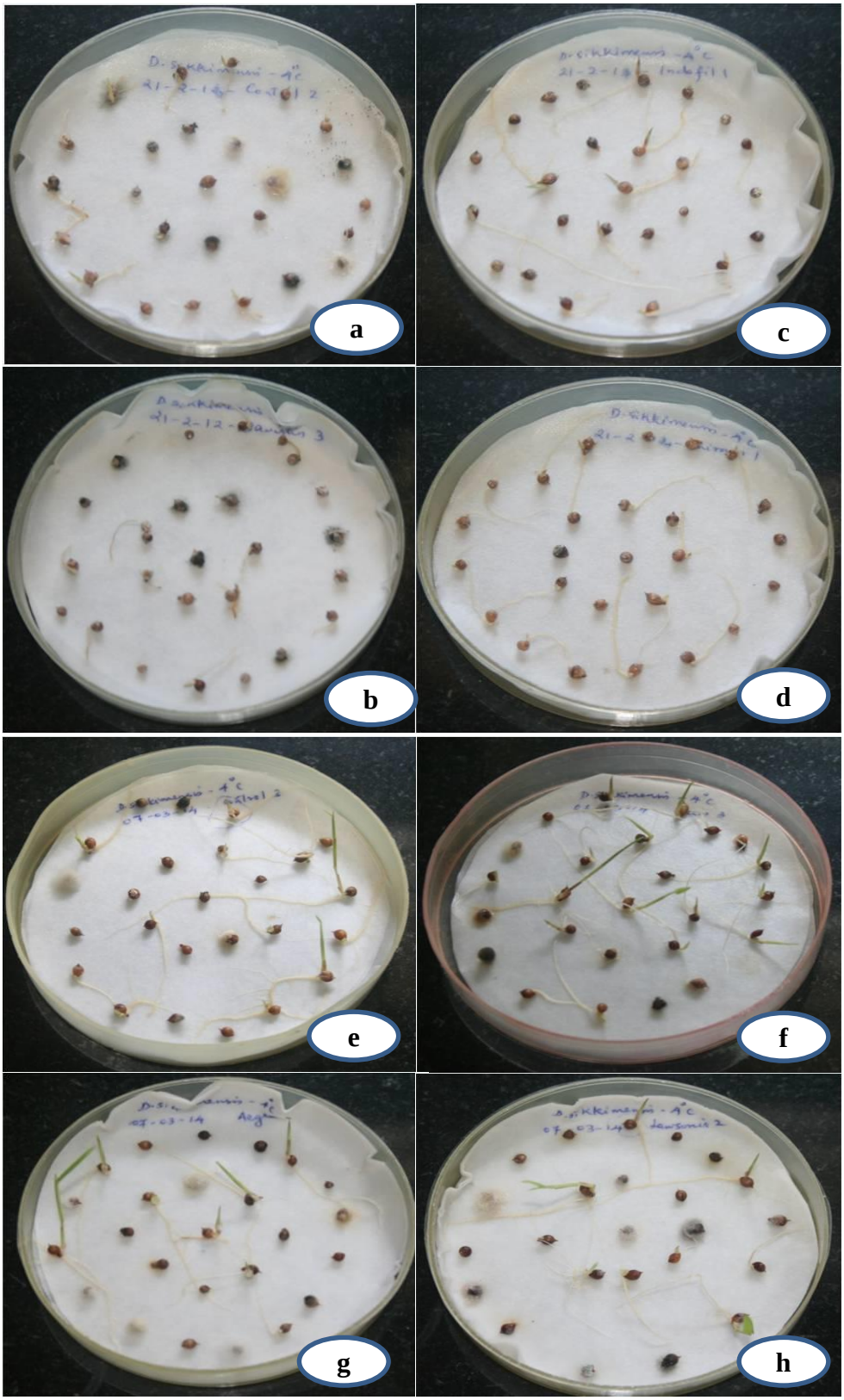

Plate 3. Effect of fungicides and biologicals on seed mycoflora :
Fungicides - a. Control, b. Bavistin, c. Indofil, d. Thiram
Biologicals - e. Control, f. Neem, g. Aegle, h. Lawsonia

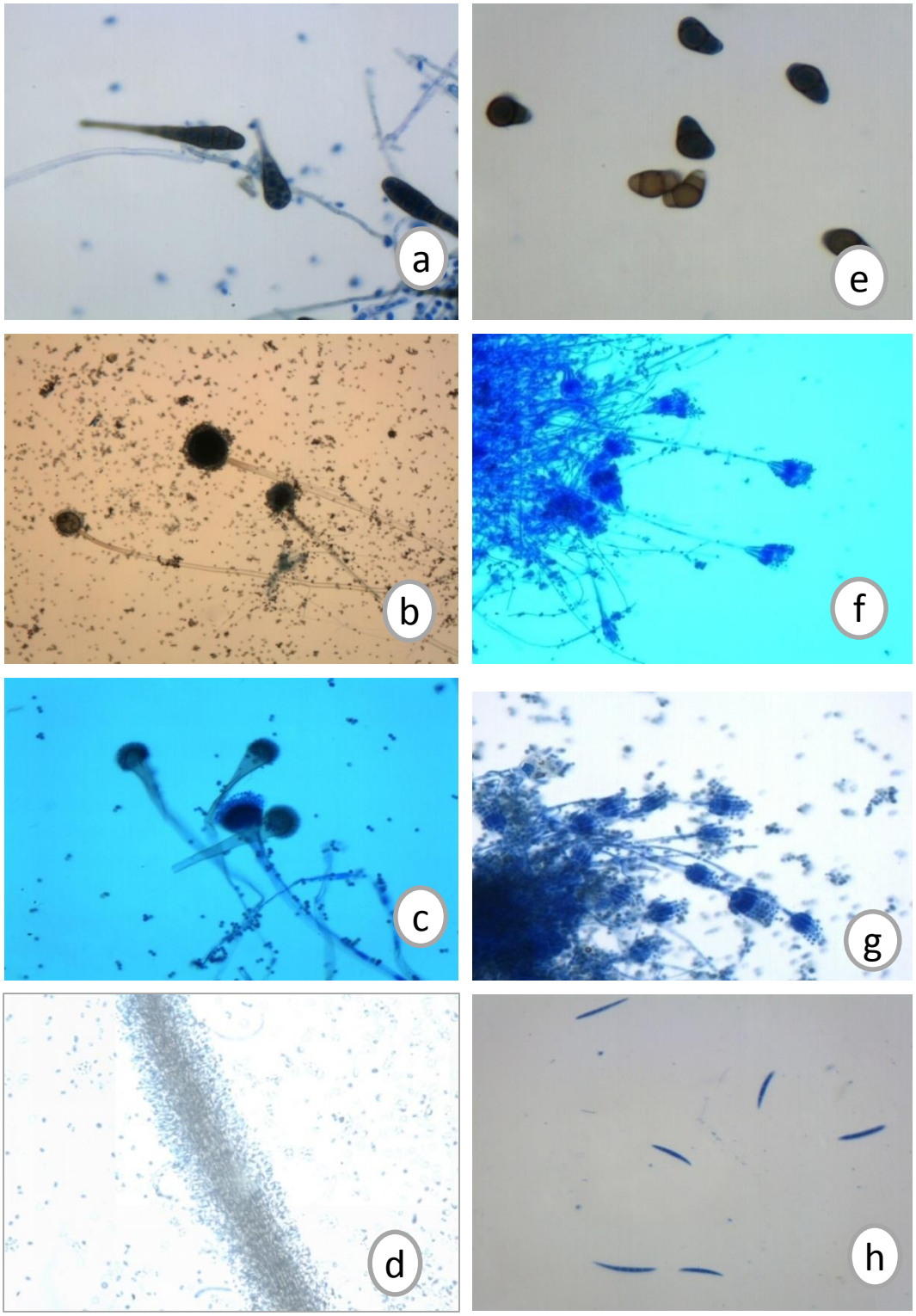

Plate 4. Seed mycoflora: *a. Alternaria tenuissima*; b. *Aspergillus niger*; c. *Aspergillus fumigates*; *d. Stysanus sp.*; *e. Curvularia lunata*; *f. Pencillium chrysogenum*; *g. P. sclerotiorum*; *h. Fusarium oxysporum*