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A study on variations in viability of *Syzygium caryophyllatum* (L.) alston seeds stored at various conditions

Chithra S G[1], Neethu S Kumar[2], Santhoshkumar R[3]
[1] Post Graduate Department and Research Centre of Botany, Mahatma Gandhi College, Kesavadasapuram, Kerala, India
[2] Department of Botany, HHMSPB NSS College for Women Neeramankara, Thiruvananthapuram, Kerala
[3] Department of Botany, NSS College, Pandalam, Kerala

Abstract
Storage of seeds under controlled environmental conditions helps to maintain seed viability for a longer period and it also depends on initial seed quality, moisture content and temperature during storage. Reduced rate of moisture content and temperature will affect the viability of seeds. *Syzygium caryophyllatum* (L.) Alston is a very significant evergreen shrub or tree which belongs to family Myrtaceae. Its seeds are known to be recalcitrant. The present study, is mainly focussed to identify the variation in moisture content, germination percentage and viability of seeds stored at various storage conditions and to report a suitable storage method for *Syzygium caryophyllatum* seeds. The collected seeds were stored at ten different conditions and were analysed at regular intervals. The seeds were found to have a maximum viability period and germination percentage when stored at refrigerator along with fruits compared to other conditions. Inappropriate storage medium such as room temperature storage often results in low seed germination, seed deterioration, and loss of viability, which are natural phenomenon during seed storage.

Keywords: *Syzygium caryophyllatum*, recalcitrant, seed deterioration

Introduction
Syzygium caryophyllatum (L.) Alston belongs to family Myrtaceae. In Hortus malabaricus it is mentioned as njara by Van Rheed (1678-1703) [16, 12]. It is placed in Endangered under the Red list category of IUCN Red list of Threatened species [25]. The tree bears a globose fruit which is pale green on unripe condition and turn to purplish black upon maturity. After maturation, recalcitrant seeds shed from the tree with a higher rate of moisture content and metabolic activity but with poor storage potential [9, 19]. In recalcitrant seeds, loss of seed viability may be due to various factors and has been categorised into three general sections which include mechanical damage, metabolism induced damage and macromolecular denaturation [18]. The most suitable way to diminish the rate of all the three types of damages is by maintaining an appropriate storage temperature. Preservation of seeds at suitable temperature help to constrain the rate of moisture loss thereby eventually reducing the damage [3, 8, 11, 15]. Storage of seeds at lowest suitable temperature is appropriate but at the same time care should be taken that it has not been affected by chilling or freezing injury. The tolerance of seeds with respect to storage at lowest temperature may vary with species. Seed moisture content and temperature are the major factors for the deterioration of seeds during storage [14]. During the progress of seed ageing, there occurs various changes in physiological and biochemical factors which may enhance ultimately resulting in the loss of seed viability [2, 4, 7, 10, 17, 24].

Materials and Methods
The plant materials were collected from Nanniyode,Palode, Thiruvananthapuram, Kerala. The plants were identified with the help of Flora of Presidency of Madras (Gamble and Fisher, 1928). Healthy and matured seed samples were collected on the basis of the time of harvest, colour of the fruit etc. Seeds were collected by extracting the fruits picked from the ground, either by looping the branches or by manual shaking. The collected seed samples were shade dried, cleaned thoroughly to remove foreign materials and damaged seeds and stored in air tight containers.

Storage of seeds at different conditions
Seeds were stored under ten conditions.
Storage condition 1: Stored at Room temperature (seeds with fruits & seeds without fruits)
Storage condition 2: Stored inside the Refrigerator (seeds with fruits & seeds without fruits)
Storage condition 3: Stored inside Freezer (seeds with fruits & seeds without fruits)
Storage condition 4: Stored inside Glass bottles (seeds with fruits & seeds without fruits)

Storage condition 5: Stored inside a Dessicator containing silicagel (seeds with fruits & seeds without fruits)
Seeds were packed in sterilized cotton cloth and then stored under the above mentioned conditions. About 100 seeds were used for each storage condition.

Determination of moisture content
Moisture content of the seeds were estimated by taking 25 transversely cut seeds at a time in a petridish and weighed before and after incubation in a hot air oven at 80^0C for 24 hrs. Loss of weight of the sample was expressed in moisture content and also the average value of the triplicate determination were expressed on percentage basis.

$$\% \text{ of moisture content} = \frac{\text{(Fresh weight- Dry weight)}}{\text{Fresh weight}} \text{ x } 100$$

Determination of Germination Percentage
Germination percentage is simply the proportion of seeds that germinate from all seeds subject to the right conditions for growth. Five seeds from each storage condition were sowed in field containing briquette. This process of germination was repeated continuously until the germination is completely stopped in all storage conditions.
Percentage of germination was calculated using the following formula:

$$\text{Percentage of germination} = \frac{seeds\ germinated}{total\ seeds\ sowed} \times 100$$

Viability test (Tetrazolium)
Samples was taken and soaked overnight in water. After overnight incubation the seeds were cut open longitudinally and put inside a beaker. To this tetrazolium solution was added until the embryo got immersed into it. For optimum level staining, the samples were placed inside a waterbath at 40ºC for 1 hour. The solution was then drained and checked for the staining pattern.

$$\text{Percentage of viability} = \frac{\text{No.of stained embryos}}{\text{Total no. of embryos}} \text{ x } 100$$

Result and Discusssion
During day one of incubation of storing seeds with fruits, at room temperature, the moisture content was found to be 69.63%. However, the percentage of moisture content was drastically decreased after 9 days of storage with only 31 percentage of moisture, with 90% of germination during day one itself. However, the percentage of germination got significantly reduced to zero after 9 days of storage (Fig 1) In the case of storage with seeds alone, maximum moisture content of 52.09% were obtained during day one, and noticed a drastic decrease in moisture after 5 days of storage. Here 100% of seed viability were noticed during day one of storage, whereas the percentage of germination got significantly decreased to zero after 5 days of storage. (Fig 2)
Whereas in refrigerated condition , when the seeds were stored along with fruits, there was a gradual decrease in the moisture content from 5 to 15 days of storage and had shown a maximum moisture percentage of 54.65% during the first day of incubation . Also the seeds retained high moisture content till 9 days (38.41%) followed by 12 days (35.99%) and the seed viability of about 28% was obtained during 15 days of storage. In this condition of storage, a significant reduction of germination percentage was detected from 9 to 15 days of storage period. High germination percentage of 90% was noticed during the initial days. Also had shown a germination percentage of 20% on 15th day of storage (fig 3).When the seeds alone were stored at refrigerated condition, there was a gradual reduction in moisture content from 50.83% to 27.12%. In this condition, a gradual decrease in viability percentage too was detected from day one to day fifteen of storage period. In both refrigerated incubations, the germination percentage was found to be almost100% even during day one itself. The viability on initial stages were high and reduced to 20 % and had shown a germination percentage of 10 % on twelth day of incubation. (Fig 4)
In freezer storage, a gradual and slight variation of moisture content was detected from 5 to 9 days of storage period. High moisture content (55.96%) was noticed during day one of incubation. Viability was high during initial days which was reduced to 10 % from day seven onwards. It was also noted that seeds possess a germination percentage of zero after 5 days of storage. There was a, drastic reduction of germination percentage from 1 to 5 days.(Fig 5).in the second case, it was recorded only a slight variation in moisture content as incubation days progressed and the viability was noticed almost 100% on first day but had drastic reduction and became 20% on fifth day itself. Germination stops after third day of incubation. (Fig 6)
In dessicator storage of seeds along with fruits, the moisture content was high during first three days and decreased from twelve day onwards.. There was only a small difference in moisture content between day five and day 7. There was a significant and gradual reduction in viability percentage from 7^{th} day of incubation.. It was noticed that seeds had high viability percentage on day one (100%) and low in day nine (28%).There was a

significant and gradual reduction in germination percentage from 1 to 5 days of storage period. It was noticed that seeds had high germination percentage in day one (85%) which got reduced gradually. (Fig 7). Whereas in seeds alone, high moisture content on initial days were decreased after twelve days of incubation. It possessed high viability on day one and declined to 20% on ninth day. High germination of about 80% was noticed initially and became 40% on five days of incubation. And the percentage was declined to zero (Fig 8)

In glass bottle incubation of seeds along with fruits, there was a gradual reduction in moisture content on initial days and a drastic reduction from day nine onwards. There was 100% of germination on day one. But there was a decrease from day three of incubation. And the percentage declined to zero from day nine onwards .In the case of viability it was 90% on day one of storage period. But the viability had reduced from 3 to 5 days of storage. (Fig 9). In the seeds alone case the same kind of reduction was noticed as in the first case, with an initial moisture content of 57.33%. 100 % of viability was on initial days whereas the percentage of germination got decreased as incubation days progressed. In both conditions the germination stopped from 7th day of storage onwards. (Fig 10)

Reports suggest that as moisture content declines the germination rate is also diminished [13, 21, 26, 29]. When the seeds of Hopea parviflora Beddome and Hopea pomga (Dennst) Mabb. are dried to a moisture content of 26% and 28%, they showed a reduction in the rate of germination potential [22]. Asomaning *et.al*[1] studied in the seeds of Garciniakola and recorded a maximum of 80% germination when the moisture content was 58% and reported a reduction to 100% germination when the moisture content was deducted to 25-27%. Irrespective of the species, lower temperature helps to maintain seed viability potential than ambient temperature, but when it extends beyond a limit, the low temperature will cause a negative effect on viability [27]. Temperature above 0° C has accelerated the total loss of viability in mango[5] (5-10°c), mangosteen[28] (10°c) and rambutan[6] (6°c). Storage at sub-zero temperature will also activate the rate of viability loss which was suggested by Roberts [23] and Pritchard [20] et al.

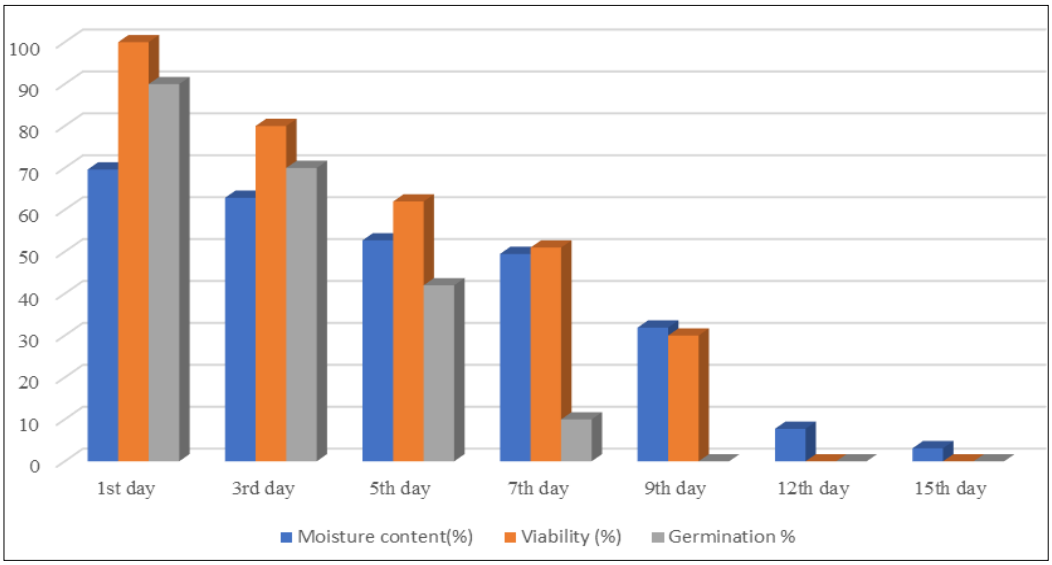

Fig 1: Room temperature-seeds with fruit

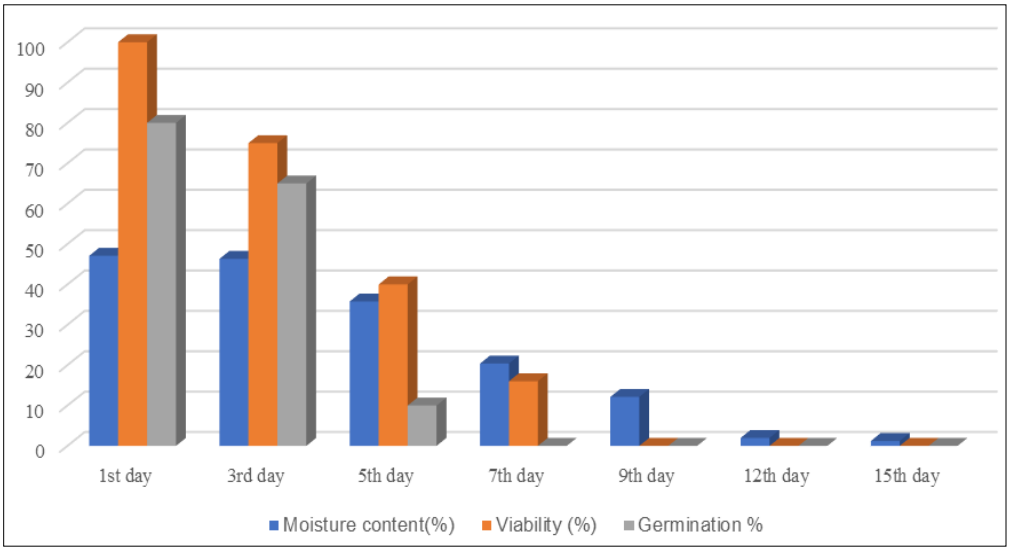

Fig 2: Room temperature-seeds alone

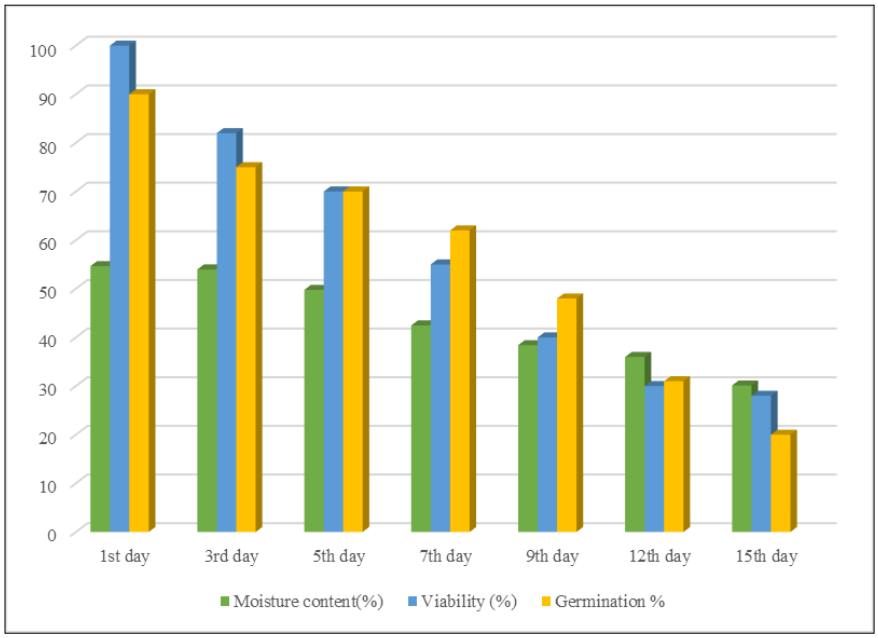

Fig 3: Refrigerator-seeds with fruit

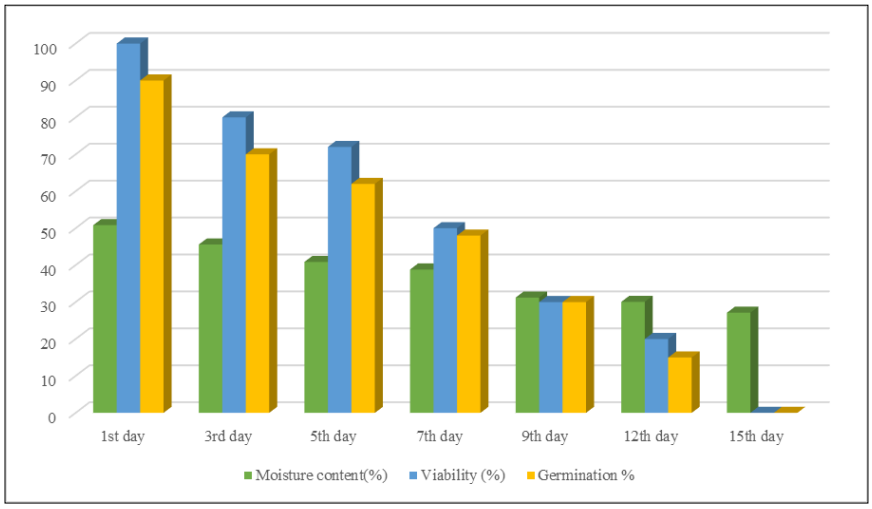

Fig 4: Refrigerator -seeds alone

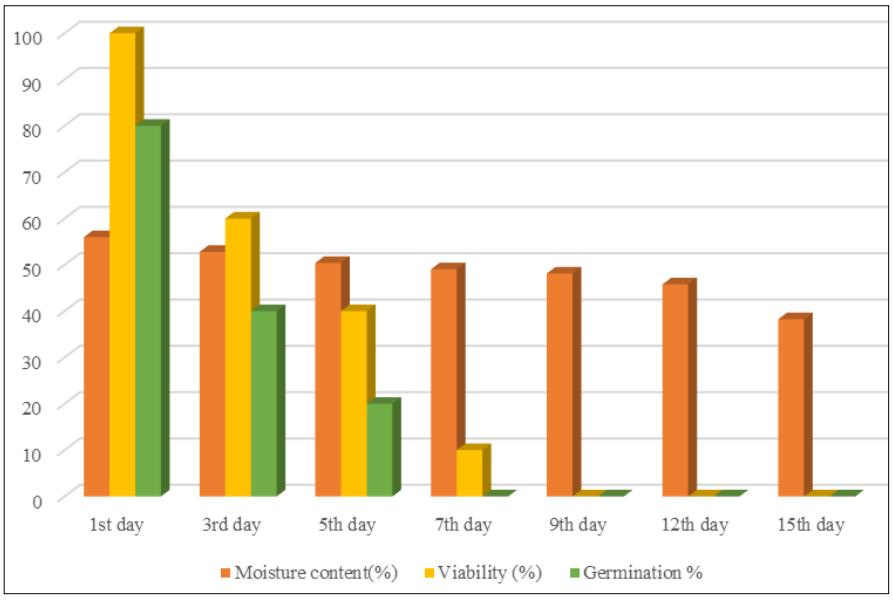

Fig 5: Freezer- seeds with fruit

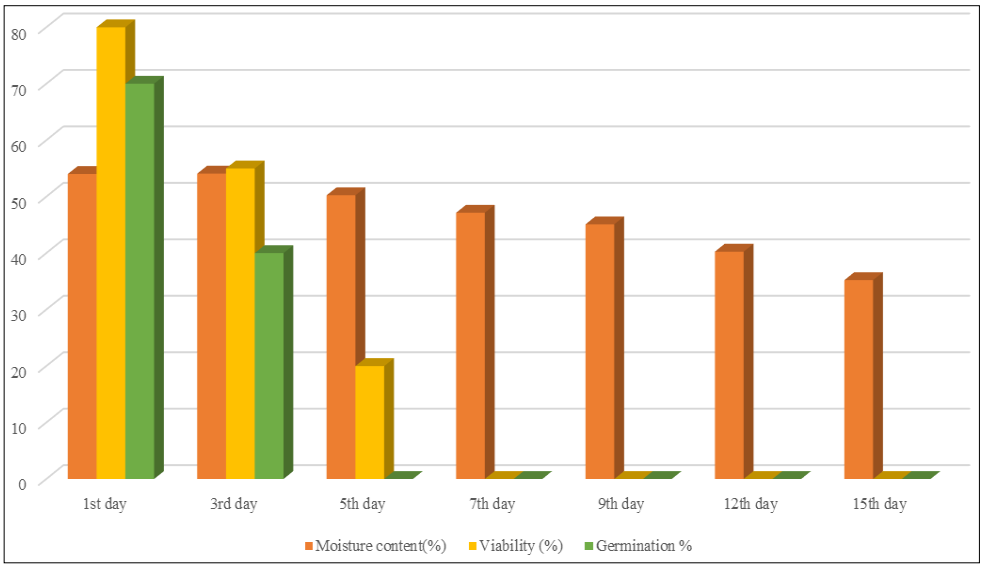

Fig 6: Freezer- seeds alone

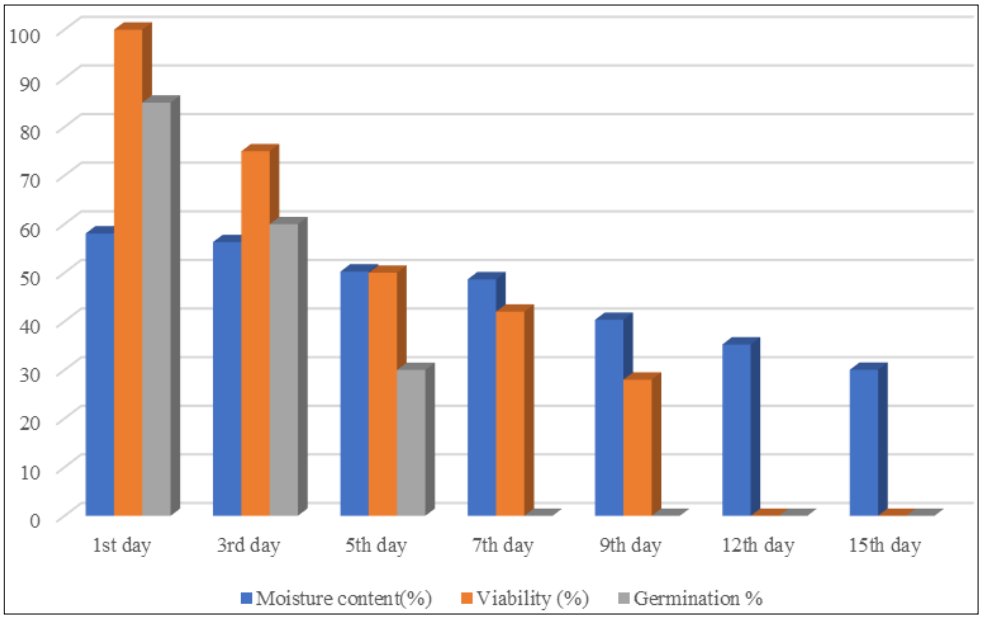

Fig 7: Dessicator- seeds with fruit

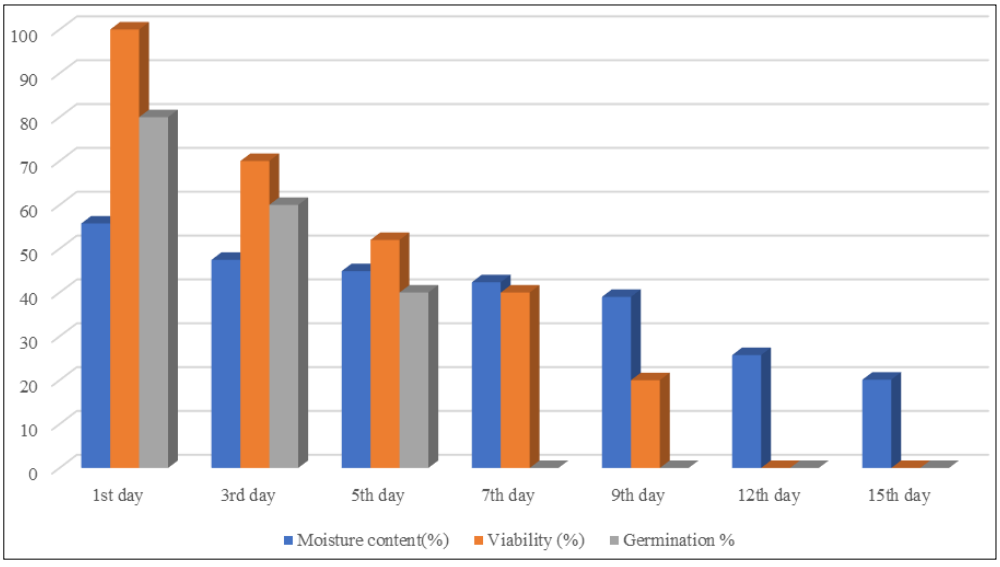

Fig 8: Dessicator- seeds alone

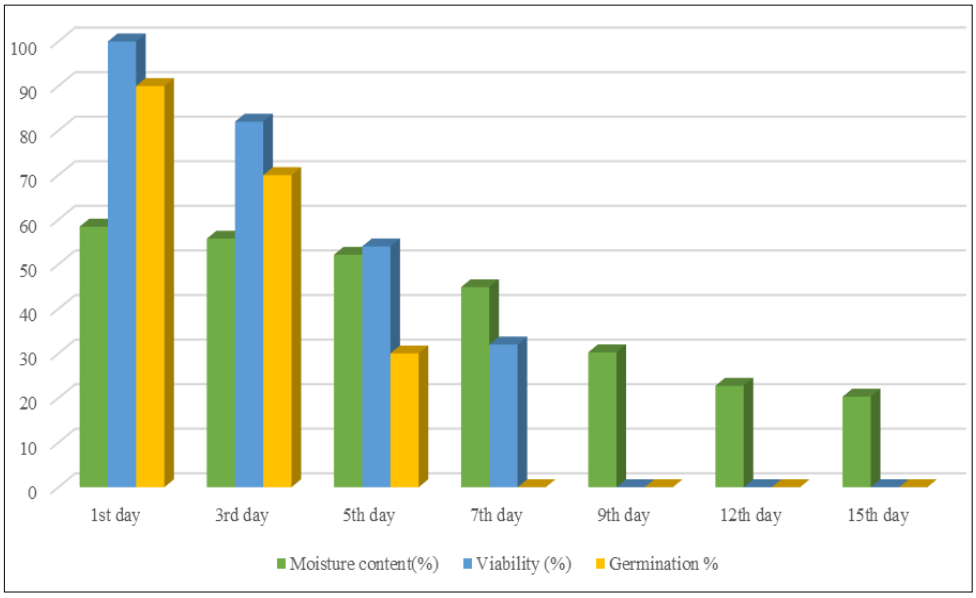

Fig 9: Glass bottle – seeds with fruit

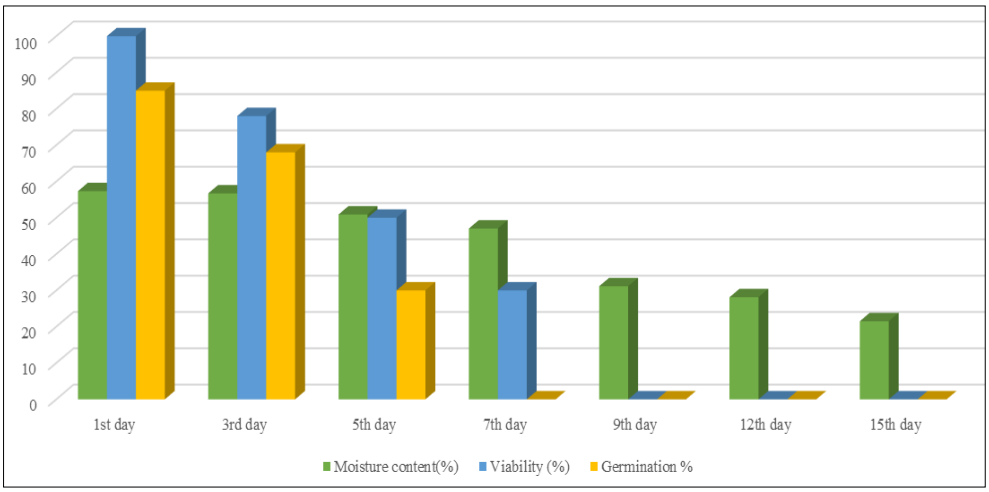

Fig 10: Glass bottle- seeds alone

Conclusion

During storage the quality of seed is persuaded by several factors and there is a rapid downturn in growth of seedling, germination capacity and resilience to adverse conditions. As moisture content reduces, seeds start decaying which ultimately leads to the death of the recalcitrant seeds. The most applicable way to store the recalcitrant seed is by depositing them at lowest suitable temperature so as to diminish the rate of damages.

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