

## Effect of Developmental Stages on Neem (*Azadirachta indica*) Seed Storage



**Oblé Neya, Christine S. Gaméné and  
Lambert G. Ouédraogo**

Centre National de Semences Forestières,  
01 BP 2682 Ouagadougou 01, Burkina Faso

**Jean B. Ilboudo**

Université Polytechnique de Bobo Dioulasso (IDR),  
01 BP 1091 Bobo Dioulasso, Burkina Faso

**Moctar Sacandé**

Seed Conservation Department, Royal Botanic  
Gardens, Kew, Wakehurst Place, Ardingly, West  
Sussex RH17 6TN, UK



### Summary

Neem seeds from various locations show variable responses to desiccation, but less is known about how maturity affects neem seed storage performance in the dry state. Seeds collected in Burkina Faso at three different developmental stages were stored in the seed laboratory of CNSF (Ouagadougou) at various temperatures (25°C in a cabinet, 4°C in a cool room and -18°C in a freezer) and different moisture contents (36–42%, 12%, 8% and 4%). Some seeds of all three developmental ages showed sensitivity to dehydration to the lowest moisture content, with viability falling to around 40–70% from c. 80–100%. During storage for up to 6 months, highest survival was observed for seeds from the last collection subsequently held at near full hydration and at 25°C, and seed longevity at 36–42% ≥ 4% ≥ 7% ≥ 12% moisture. At 4°C and -18°C, the viability of the driest seeds (4% moisture) was relatively constant throughout storage, whilst it decreased rapidly for wetter samples, presumably as a result of chilling and freezing injury. The results suggest that the benefits of cold dry storage over wet warm storage would become evident in neem seeds after 6 months storage.

## Introduction

The neem is a tree species which was introduced to the African continent from Asia at the beginning of the nineteenth century (Dewaulle, 1977). It is an excellent shade tree, widely used in courtyards, in public parks and in the demarcation of fields (Sacandé, 1995). In addition, its products have many different uses. The wood is used in sculpture and in the fabrication of carts, tools and coffins (Devernay, 1994). Oil extracted from the seed is traditionally used in India as a lubricant for cartwheels and as lamp oil (CTFT, 1988; Devernay, 1994). The seeds are now also widely used in the pharmaceutical and veterinary industries, for the extraction of nimbidine, and antiseptic soaps based on neem extracts are made in Burkina Faso (Neya, 1999). The importance of this exotic species, which has become naturalised in the sudano-sahelian region (Ganaba, 1996), for mankind and his environment is beyond doubt.

Neem is mainly propagated by seed. However, multiple collecting operations on different sites in originating countries, with depulping before or after transport, drying on the spot and timely dispatch to other tropical areas has never been planned on a large scale (Bellefontaine, 1992). This is due to the difficulties local researchers have with respect to conserving seeds of this species.

Publications on the physiology and conservation of neem seed often describe contradicting results. Ezumah (1986), CNSF (1993), Gunasena and Marambe (1995) and Msanga (1996) concluded that neem seeds lose their viability after

one to four months of storage and have consequently classified them as recalcitrant. Roederer and Bellefontaine (1989), Dickie and Smith (pers. comm.) showed that neem seeds survive progressive desiccation to a moisture content of about 4% (fresh mass basis) and long-term storage (8 to 10 years) in hermetically-sealed containers at a temperature of either 4°C or -20°C. These authors concluded that neem seeds are orthodox. Other studies undertaken by Gaméné *et al.* (1996) and Sacandé *et al.* (1996) showed that neem seeds are intermediate in seed storage behaviour when compared with orthodox and recalcitrant seeds. These clear differences in the ability of seeds of this species to retain viability might be due to factors such as provenance, storage conditions and seed developmental stage.

The main objective of the study presented here was to determine the possible impact of fruit developmental stage on the storage longevity of neem seeds. Effects of storage temperature and seed moisture content on viability were also taken into account.

## Materials and Methods

Seeds were extracted from neem fruits collected at 7, 8, 9 weeks after presumed fertilisation. Fruits were harvested in Ouagadougou, in the Sudanian zone of Burkina Faso, following field monitoring of fruit development to estimate the approximate age of each seed lot.

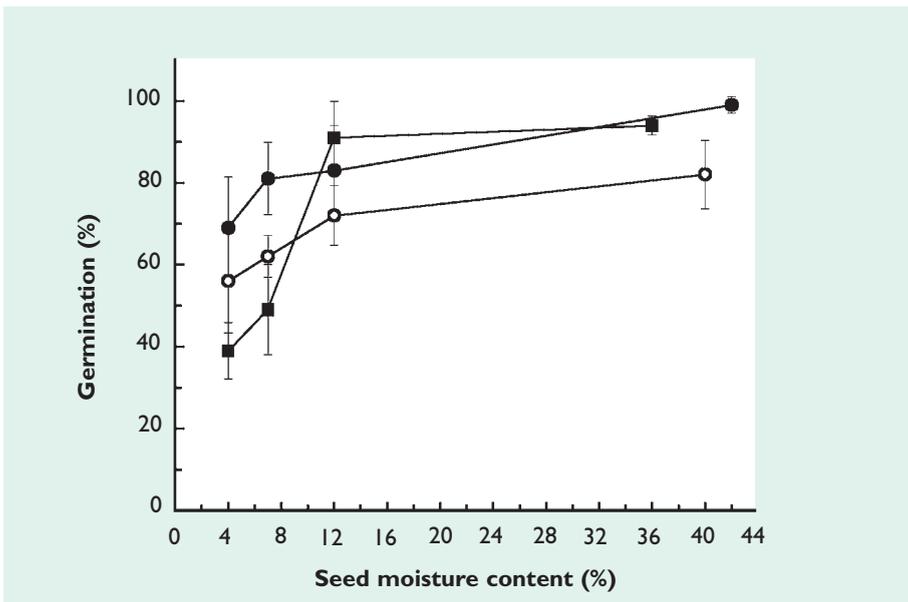
Storage trials were carried out over 6 months on seed lots with four different moisture contents (freshly harvested and three desiccation treatments). The dried seeds were obtained by dehydrating fresh seeds over silica gel to 12%, 8% and 4% moisture content (fresh mass basis). Seeds extracted from 7, 8 and 9 week-old fruits had initial moisture contents of 42%, 40% and 36% respectively. After drying, the seeds were further divided in three sub-lots, packed into aluminium bags and then placed in three different conditions: a cabinet with a temperature of 25°C; a cooled room with a temperature of 4°C; and a freezer with a temperature of -18°C. Every month, the bags were opened and samples were removed. The bags were then resealed and returned to storage. The samples were used to determine seed moisture content and germination capacity in order to monitor seed quality over time.

For moisture content determination, five replicates of five seeds were weighed before and after drying for 17 h in an oven at 103°C (ISTA, 1999). The results are presented as a percentage of fresh mass. For germination, 4 × 25 seeds were sown on moist filter paper and incubated at c. 25°C under natural light levels. Germination was assessed as radicle emergence to c. 5 mm, and the tests were monitored twice a week.

## Results and Discussion

### 1. The Effect of Maturity on Desiccation Tolerance

Figure 8.1 shows the effect of seed maturity on the tolerance of dehydration. The initial level of germination varied slightly between maturity stages, ranging from 82 to 98% (Figure 8.1). For all three harvest times, seeds tolerated drying to 12% moisture content with little change in germination level (Figure 8.1). Drying to 7% moisture content generally resulted in a decrease in viability. Further dehydration to 4% moisture content resulted in about a 30–50% lower germination level than the fresh control (Figure 8.1). Nonetheless, 40–70% of the seeds, depending on harvest time, survived drying to this lowest moisture content.



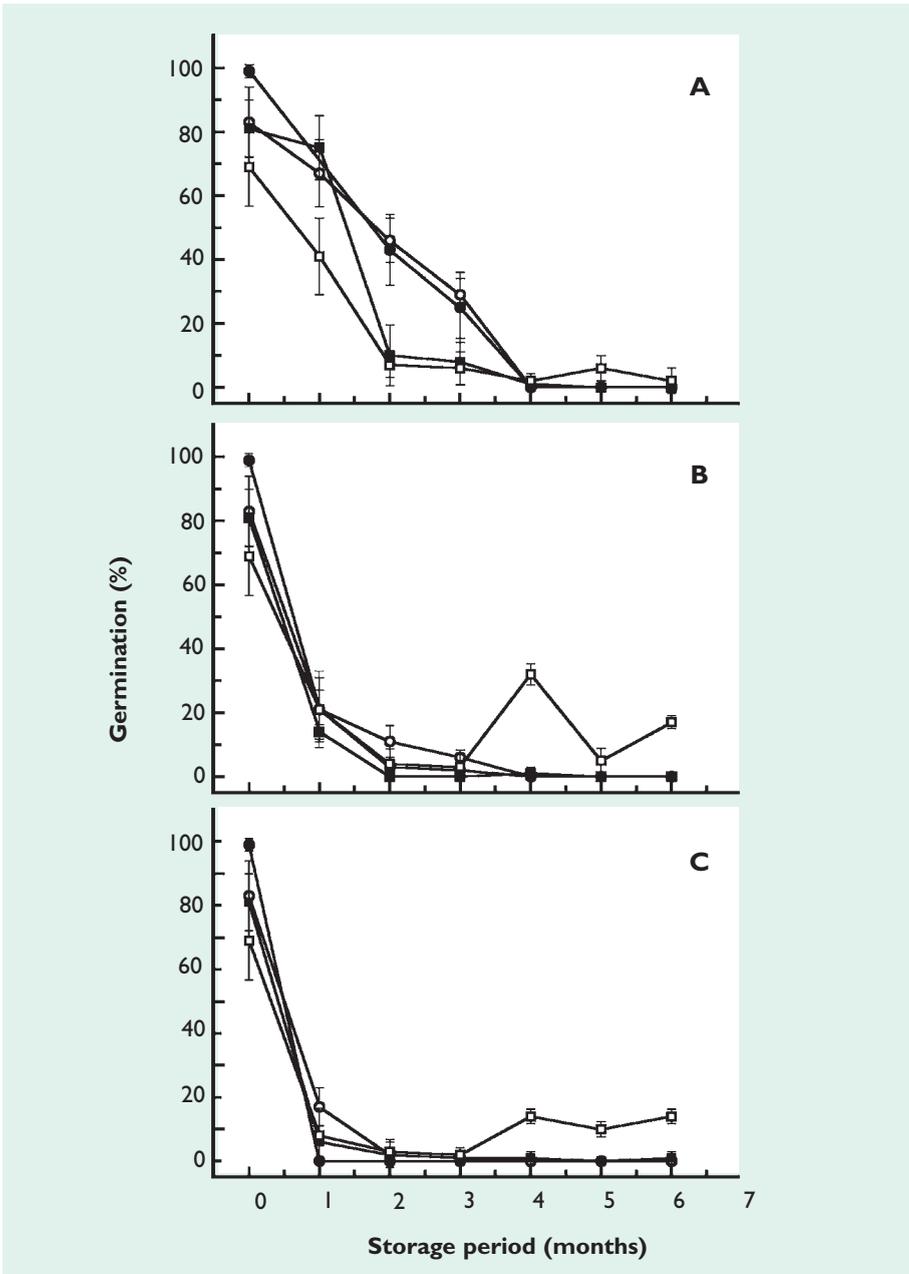
**Figure 8.1** Effect of desiccation on germination of neem seeds during development. Seeds were extracted from fruits harvested 7 (●), 8 (○) and 9 (■) weeks after flowering of the trees.

Drying neem seeds to c. 7% moisture content and below probably induces the formation of the glassy state (Sacandé, 2000), whereas seeds at 12% moisture are still non-glassy. Whilst this might suggest that desiccation sensitivity in some seeds is negatively associated with the formation of the glassy state, Sacandé (2000) has proposed that it is rehydration rather than dehydration that contributes to this type of response. When dry neem seeds are dried to equilibrium with about 50% relative humidity (c. 4% moisture content), imbibitional stress can only be overcome by soaking the seeds at an elevated temperature prior to the germination test. Although this treatment was not applied in this study, it is clear that some seeds did not require such treatment in any case (Figure 8.1).

## **2. The Effect of Seed Age and Storage Temperature on Seed Longevity at Different Moisture Contents**

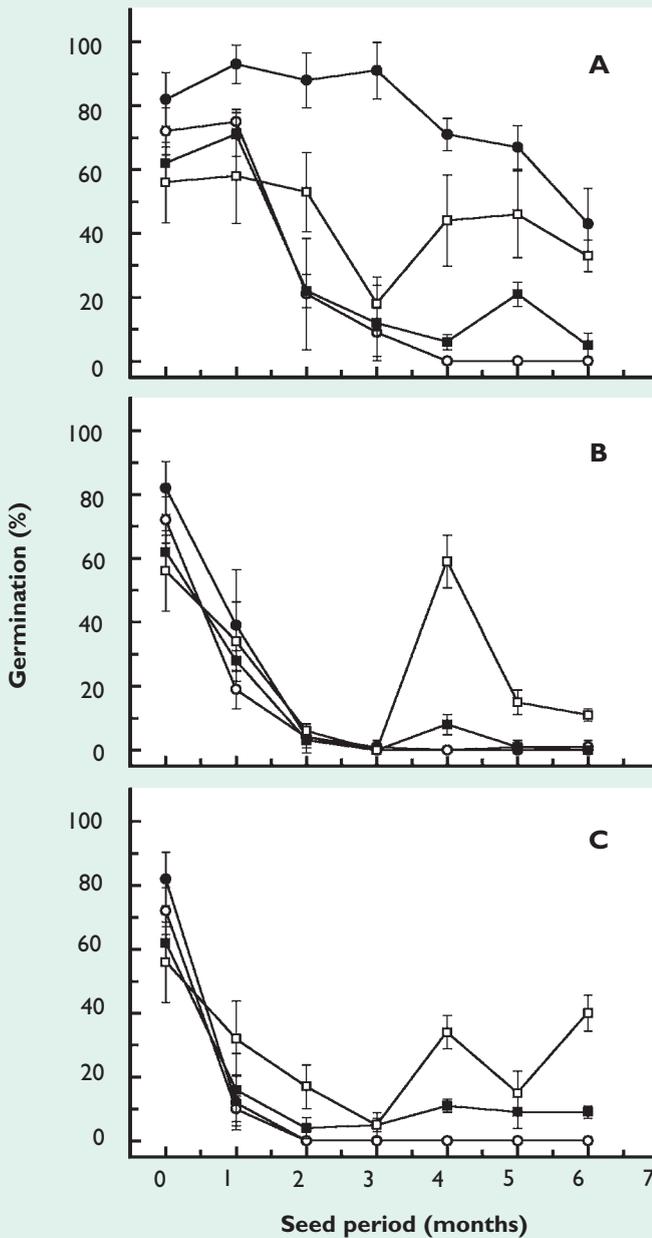
The effects of seed age on storability are shown in Figures 8.2, 8.3 and 8.4. Seven week old seeds retained c. 30% germination after 3 months at 25°C when stored at 12 or 42% moisture content. However, no seeds survived 6 months at this temperature, and the drier seeds had shorter longevity than the wetter ones (Figure 8.2A). Generally, seeds of this age at all the moisture contents tested, stored less well at 4°C and -18°C than at 25°C (Figures 8.2B and C). At both temperatures, little viability remained after the first month of storage. This was presumably as a result of the induction of chilling injury at 4°C and chilling plus freezing injury, depending on moisture content, at -18°C. Nonetheless, a few (10–20%) of these immature seeds survived six months storage at the lowest moisture content (4%) (Figures 8.2B and C). This suggests that cold injury is primarily operable when seeds are hydrated to  $\geq 7\%$  moisture content. From the sorption isotherm in Sacandé (2000), this level of hydration equates to about 60–70% RH.

There was evidence that the longevity of eight week old seed at 25°C and most moisture contents was better than seven week old seed at the same temperature (Figure 8.3A). The wettest (40% moisture content) and driest (4% moisture content) seed retained c. 30–40% germination after 6 months storage; the intermediate moisture content seeds were shorter lived. One interpretation of this data is that the fully hydrated seeds were capable of repair during storage as previously observed for seeds of other species, thereby enhancing their longevity (Villiers and Edgecumbe, 1975; Roberts and Ellis, 1989). In contrast, intermediate moisture content (7 and 12%; c. 65–85% RH) seeds were wet enough to age rapidly but too dry for effective repair to occur. When eight week old seeds were stored at 4°C, only the driest seed survived 6 months storage, but only to c. 20% (Figure 8.3B). A similar situation occurred for seeds stored at -18°C after 6 months, although survival was 40% for the driest seeds and a few seeds at 7% moisture content also survived. There was no survival for the wettest seeds. As for the seven week old seeds, these seeds also only avoided the ravages of cold injury when at the lowest storage moisture content.



**Figure 8.2**

Effect of temperature and moisture content on storage of neem seed extracted from fruits harvested 7 weeks after flowering of the trees. Seeds were stored at 25°C (A), 4°C (B) and -18°C (C) and at 42% (●), 12% (○), 7% (■) and 4% (□) moisture content. Bars represent the standard deviation of the mean, based on a sowing of 4 × 25 seeds.

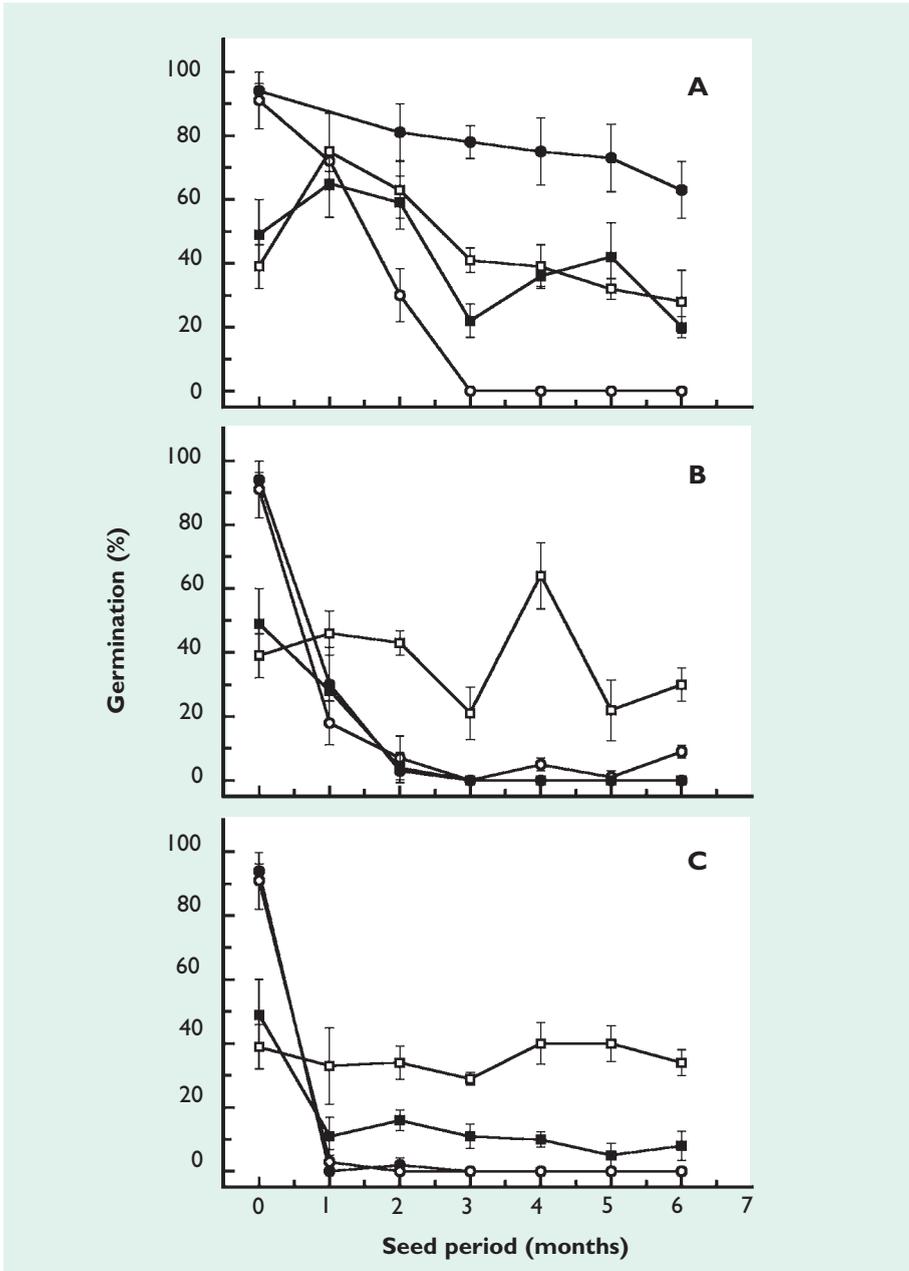


**Figure 8.3** Effect of temperature and moisture content on storage of neem seed extracted from fruits harvested 8 weeks after flowering of the trees. Seeds were stored at 25°C (A), 4°C (B) and -18°C (C) and at 40% (●), 12% (○), 7% (■) and 4% (□) moisture content. Bars represent the standard deviation of the mean, based on a sowing of 4 × 25 seeds.

The results for nine week old seeds, were similar to those for eight week old seeds, the exceptions being generally improved performance of the driest seeds when stored at 4°C and -18°C (Figures 8.4A, B and C). Similarly, Esswara *et al.* (1998) observed that fully ripe neem seed from the drier regions of Sri Lanka only tolerated storage in the freezer at < 10% moisture content. One problem associated with wet storage at warm temperature was fungal contamination of the seed. Precautions should therefore be taken to dress the seeds with fungicide before storage under such conditions. In addition, the cool storage results for the wet seeds are comparable to those of Sacandé *et al.* (1998), who concluded that neem seeds from Burkina Faso and Sri Lanka with moisture contents between 33 and 38% completely lose their viability within 1 to 3 months storage c. 5°C.

Overall, the preservation of viability of stored neem seeds improves with maturity, as the fruits from which the seeds were extracted changes from green to yellow. As a consequence, we advise collection of fruits no earlier than eight weeks after flowering. The results and recommendation are in accordance with those of Gaméné *et al.* (1996) and Sacandé *et al.* (1996); and Sacandé *et al.* (1996; 1998). Moreover, warm temperature and high moisture contents appear to offer better storage conditions than colder, drier conditions. For example, the highest germination after six months storage was observed in nine week old seeds held at c. 36% moisture content and 25 °C. These seeds retained c. 60% germination (Figure 8.4A). These results support earlier reports of Gaméné *et al.* (1996) and Yaméogo (1997). Nonetheless, wet seeds progressively lost viability (see Figures 8.3A and 8.4A), whilst the driest seeds stored at -18°C maintained viability, albeit at a lower level (30–40% ) than for the wet seeds over six months (see Figures 8.3B and 8.4 B). There are two notable aspects of the results. Firstly, that the dry, cold stored seeds should outperform wetter stored seeds over the longer-term. In other words, the most mature seeds show an orthodox type of storage behaviour. Secondly, only moderate levels of survival at dry, cold conditions suggest that even the most mature seed lot was heterogeneous. This heterogeneity might be due to variation in flowering time in relation to the mother-trees and/or the collection sites.

In conclusion, the effects of storage moisture content and temperature on neem seed longevity are highly dependent on the maturity of the seed, with the maturest seeds exhibiting an orthodox-type response.



**Figure 8.4**

Effect of temperature and moisture content on storage of neem seed extracted from fruits harvested 9 weeks after flowering of the trees. Seeds were stored at 25°C (A), 4°C (B) and -18°C (C) and at 36% (●), 12% (○), 7% (■) and 4% (□) moisture content. Bars represent the standard deviation of the mean, based on a sowing of 4 × 25 seeds.

## Acknowledgement

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