GERMINATION RESPONSES OF *KHAYA ANTHOTHECA* SEEDS TO A RANGE OF ALTERNATING AND CONSTANT TEMPERATURES PROVIDED BY THE 2-WAY GRANT'S THERMOGRADIENT PLATE

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ABSTRACT

Khaya anthotheca seeds were placed in Petri dishes containing a gel of 1% water agar for germination over a period of 30 consecutive days. Petri dishes were arranged 8 units x 8 units on the 2-way Grant's thermogradient plate (a bi-directional incubator). The goal of the study was to take a more comprehensive look at germination responses of seeds to a broad range of alternating or constant temperatures on the thermogradient plate. The instrument allows for germination testing of seeds over a wide range of single temperature and alternating temperature regimes over a time continuum, given 64 temperature combinations (regimes) (5 to 40°C). Conditions were 40/40°C (day/night temperature) on the high end of the plate and 5/5 ºC on the cool end. Two temperature gradients ranging from 5 to 40°C were used. The first gradient, progressing from left to right on the thermogradient plate in dark, was alternated every 12 hours with the second progressing from front to back of the thermogradient plate with light. The study was repeated twice. Fifteen (15) seeds were used in each replication. The various temperature combinations had significant effect on final germination percentage, mean germination time, time for first germination and rate of germination. Alternating temperatures improved overall germination. The best germination at a constant temperature was at 20/20°C and 30/30°C. The best temperature regimes for seed germination at alternating temperatures were at 5/30°C, 10/30°C,15/30°C, 20/25°C, 20/35°C, 25/15°C, 25/20°C, 25/30°C, 30/20°C, 35/10°C, and 35/15°C.

Keywords: ***Khaya anthotheca*, germination testing, thermogradient plate, temperature regimes, germination percentage**

INTRODUCTION

Khaya anthotheca belongs to the family Meliaceae (Irvine, 1961). It is a priority species in Ghana occurring in lowland evergreen forests. The species is heavily exploited in Ghana (Hawthorne and Gyakari, 2006) and in East and West Africa and serves as a source of Africa mahogany, used in cabinet and furniture making, veneer, paneling, boat building and joinery (Irvine, 1961). The species is threatened by habitat loss, and on the 2002 IUCN Red list of Threatened species it is listed as vulnerable and therefore requiring urgent attention (Jɵker, 2003).

There is little published information on the germination characteristics and requirements of native trees seeds in the tropics. Temperature affects the germination capacity (germinability), the germination rate, and the distribution of the relative frequency of germination along the incubation time (Labouriau 1978). Studies of temperature effects on seed germination are usually limited to relatively few different temperatures because of restrictions imposed by the number of available germinators for simultaneous experiments (Larsen, 1965). The Grant thermogradient plate is a bi-directional incubator (Manger, 1999).The instrument allows for germination testing of seeds over a wide range of single temperature and alternating temperature regimes over a time continuum (Tarasoff *et al*., 2005). Within a predetermined range this device simultaneously provides all possible alternating and constant temperature combinations (Larsen *et al*., 1973).

A more comprehensive look at the germination response of the species to a broad range of alternating or constant temperatures is important so far as its ex-situ conservation and its use in afforestation and reforestation pragrammes is concerned. The present research was conducted to ascertain optimum germination requirements as well as all possible alternating and constant temperature combinations for the germination of the species.

MATERIALS AND METHODS

Seed Materials

Matured fruits of *Khaya anthotheca* were obtained from forest within the Kwahu Mountains (in the Eastern Region) of Ghana in January, 2005. The fruits (capsules) were spread on jute sacks in the sun to split open (Turnbull, 1975). Seeds were removed from opened capsules, cleaned of all debris (FAO, 1985) and dried under shade on the laboratory bench for 2 days after which they were packed in cotton bags. Seeds were immediately sent by air to the Seed Conservation Department of the Royal Botanic Gardens, Kew, in the United Kingdom where the experiment was conducted.

Seed Moisture Content and Equilibrium Relative Humidity (eRH) Determination

On receipt, seed equilibrium relative humidity at 20ºC was measured using a Rotronic AW-14P water activity measuring station (Rotronic Instruments, UK, Horley) set up with a DMS 100H humidity sensor. The essence of this experiment was to measure the relative humidity of air above the seed samples. This was to determine how dried seed samples were on receipt at the laboratory and help inform post-harvest handling of the seeds (Yang *et al*. 2003; MSBP 2005).

Seed moisture contents of the species was determined on whole seeds; five replicates of 10 seeds each was weighed before and after drying at 103ºC for 17 hours (ISTA, 1999). The moisture content was then calculated using the formula: (IW-FW)/ IW x 100.
where IW = initial weight and FW= final weight.

Seeds were dried in silica gel by mixing them on a 1:1 ratio overnight to reduce its moisture content to 6.7% after which seed samples were sealed in aluminium foil laminate bags and held at a temperature of 15ºC and 75% relative humidity until use two months later (Table1).

Germination of Seeds on the 2-Way Grant's Thermogradient Plate

Seeds were germinated on a gel of 1% water- agar in 90mm (9cm) plastic Petri dishes. Each Petri dish contained approximately 40ml of agar as recommended by (Manger, 1999). Petri dishes were arranged 8 units x 8 units on the thermogradient plate given 64 temperature combinations (regimes) (5 to 40°C). Conditions were 40/40°C (day/night temperature) on the high end of the plate and 5/5 ºC on the cool end. Dried out agar in Petri dishes located at the hot end of the plate were replaced periodically.

Two temperature gradients ranging from 5 to 40°C were used. The first gradient, progressing from left to right on the thermogradient plate in dark, was alternated every 12 hours with the second progressing from front to back of the thermogradient plate with light. Seeds were subjected to 8 constant and 56 alternating temperature regimes as shown by Table 1. The study was repeated twice. Fifteen seeds were used in each replication. Normal seedlings were scored and removed as soon as radical was 1 cm long and the plumule was visible and the location and time of removal were recorded as recommended by Larsen *et al.*, (1973). Seeds remained on the thermogradient plate for 30 days and ungerminated samples were placed at 30°C for 10 days after which the cutting test was conducted to ascertain the viability of seeds which had still not germinated (IPGRI-DFSC, 2000).

The final germination percentage (FGP) as an expression of the proportion of seeds with germination capacity, the mean germination time (MGT) as an inverse expression of the overall germination speed or rate for the whole population, time to 50 % cumulative germination (T_{50}) as an indication of germination velocity , and the time for first germination which are important aspects of the germination process, informing the dynamics of the process (Bewley and Black, 1994; Silviera *et al.*, 2005) were measured.

The germination percentages at 30 days after imbibing were calculated from the total number of seeds germinated at each temperature. The mean germination time (MGT) or average time to germination (Atg in days) was calculated following Yang *et al*. (2003). MGT (Atg) = $\Sigma (t.\text{n}) / \Sigma n$where t is time in days, starting from the day of sowing, and n is the number of seeds completing germination on day t. The time for first germination, was calculated as functions of the rate of germination as done by Bannister (1990). Germination rate was calculated as the reciprocal of the mean germination time after Mattews *et al.* (2006). Results were subjected to analyses of variance (ANOVA) to determine main effects and interaction effects using GensStat Release 4.21 (Rothamsted Experimental Station, United Kingdom).

RESULTS

Seed Equilibrium Relative Humidity (eRH) and Moisture Content

Seed equilibrium relative humidity (eRH) (%) and moisture content (MC) (%) on receipt at the laboratory in the United Kingdom and after desiccation in equal weight of silica gel overnight are presented in Table 2. Equilibrium relative humidity value of 54.7% and moisture content of 14.7% of seeds of the species on receipt at the laboratory in the United Kingdom means that the seed sample could be placed in the ‘‘damp’’ seed status and that they required immediate drying to reduce the risk of fungi attack as stated by the MSBP (2005). Drying the seeds in silica gel overnight lowered the eRH to 35.5% with seed moisture content of 6.7%, and this placed them in the ‘‘dry’’ seed status (MSBP, 2005) making them safe from fungi attack before the germination experiment two months later.

Table 1: Layout of the arrangement of treatments on the thermogradient plate

H1(40/5)	**H2**(40/10)	**H3**(40/15)	**H4**(40/20)	**H5**(40/25)	**H6**(40/30)	**H7**(40/35)	**H8**(40/40)
G1(35/5)	**G2**(35/10)	**G3**(35/15)	**G4**(35/20)	**G5**(35/25)	**G6**(35/30)	**G7**(35/35)	**G8**(35/40)
F1(30/5)	**F2**(30/10)	**F3**(30/15)	**F4**(30/20)	**F5**(30/25)	**F6**(30/30)	**F7**(30/35)	**F8**(30/40)
E1(25/5)	**E2**(25/10)	**E3**(25/15)	**E4**(25/20)	**E5**(25/25)	**E6**(25/30)	**E7**(25/35)	**E8**(25/40)
D1(20/5)	**D2**(20/10)	**D3**(20/15)	**D4**(20/20)	**D5**(20/25)	**D6**(20/30)	**D7**(20/35)	**D8**(20/40)
C1(15/5)	**C2**(15/10)	**C3**(15/15)	**C4**(15/20)	**C5**(15/25)	**C6**(15/30)	**C7**(15/35)	**C8**(15/40)
B1(10/5)	**B2**(10/10)	**B3**(10/15)	**B4**(10/20)	**B5**(10/25)	**B6**(10/30)	**B7**(10/35)	**B8**(10/40)
A1(5/5)	**A2**(5/10)	**A3**(5/15)	**A4**(5/20)	**A5**(5/25)	**A6**(5/30)	**A7**(5/35)	**A8**(5/40)

The letters A1 to H8 represent the Petri dishes (treatments) and figures in parenthesis are the 64 temperature combinations in °C experienced by each treatment during the experimental (germination) period. The zero amplitude diagonal gives a gradient of constant temperatures.

Seed Germination Percentage

The various temperature regimes provided by the thermogradient plate significantly affected final germination percentage (FGP) of the species as shown in Table 3. Germination occurred at thirty three (33) out of the sixty four (64) temperature combinations made possible by the thermogradient plate. In general seeds placed at temperature regimes with very low alternating temperatures or very low constant temperatures did not germinate (Figure 1).

Final germination percentage was highest at temperature regimes: 5/30°C (100.0%); 10/30°C (96.5%); 15/30°C (96.5%); 20/25°C (100.0%); 20/35°C (100.0%); 25/15°C (100.0%); 25/30°C (100.0%); 30/10°C (96.5%); 30/20°C (100.0%); 35/10°C (96.5%) and 5/15°C (100.0%). Final germination percentage values taken at the following temperature regimes; 5/35°C (43.5%); 10/35°C (47.0%), and 20/15°C (13.0%), were significantly lower ($p < 0.001$) than the figures recorded at the other temperature regimes. *Khaya anthotheca* seeds did not germinate at temperature regimes in which any of the alternating temperatures was 40°C.

Table 2: Equilibrium relative humidity (%) and seed moisture content (%) of *Khaya anthotheca* seed sample measured on receipt at the laboratory and after seeds were desiccated in silica gel overnight in the UK.

eRH (%) of seed samples	54.7%	eRH (%) of seed sample after desiccation in silica gel overnight	35.5%
Moisture content (%) of seed samples	14.7%	Moisture content (%) of seed sample after desiccation in silica gel overnight	6.7%

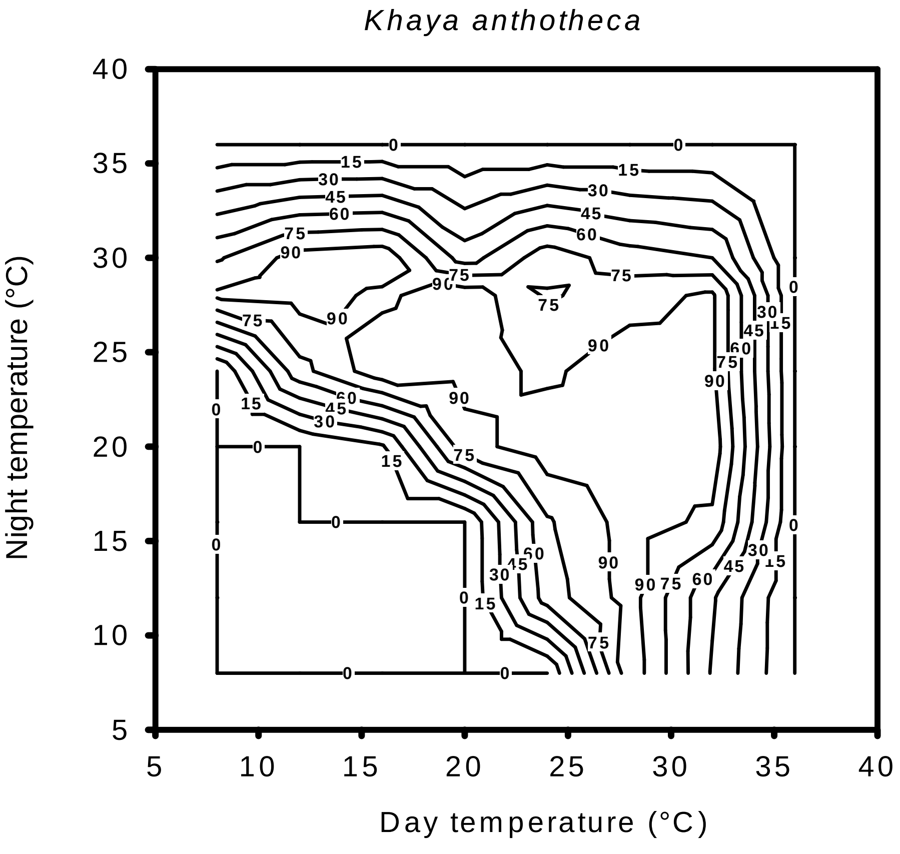

Figure 1: A map showing final germination percentages of *Khaya anthotheca* seeds on the thermogradient plate. Percentage germination values are shown on the isopleths

Table 3: Germination percentages of *Khaya anthotheca* seeds incubated at different temperature combinations on the 2-way thermogradient plate. Only temperature regimes where germination occurred are shown

Temp. (°C)	Germ. percentage (%)	Temp (°C)	Germ. percentage (%)	Temp. (°C)	Germ. percentage (%)	Temp. (°C)	Germ. percentage (%)
5/30	100.0	20/20	83.5	25/30	100.0	30/35	93.0
5/35	43.5	20/25	100.0	25/35	93.0	35/5	73.0
10/25	67.0	20/30	93.0	30/5	93.0	35/10	96.5
10/30	96.5	20/35	100.0	30/10	96.5	35/15	100.0
10/35	47.0	25/10	70.0	30/15	87.0	35/20	53.0
15/25	73.0	25/15	100.0	30/20	100.0	35/25	83.5
15/30	96.5	25/20	96.5	30/25	73.0	35/30	67.0
15/35	87.0	25/25	87.0	30/30	83.5	35/35	60.0
20/15	13.0	-	-	-	-	-	-

s.e.d = 2.547 replication = 2

d.f = 33 cv = 3.1%

Table 4: Mean germination time (days) of *Khaya anthotheca* seeds incubated at different temperature combinations on the 2-way thermogradient plate. Only temperature regimes where germination occurred are shown

Temp. (°C)	Mean germination time (days)	Temp (°C)	Mean germination time (days)	Temp. (°C)	Mean germination time (days)	Temp. (°C)	Mean germination time (days)
5/30	14.9	20/20	13.4	25/30	10.4	30/35	11.2
5/35	15.2	20/25	12.4	25/35	13.4	35/5	15.8
10/25	17.4	20/30	13.0	30/5	14.7	35/10	15.2
10/30	13.4	20/35	13.9	30/10	13.9	35/15	15.7
10/35	17.0	25/10	16.1	30/15	13.4	35/20	16.5
15/25	12.0	25/15	13.9	30/20	13.6	35/25	15.8
15/30	15.3	25/20	12.3	30/25	12.6	35/30	15.3
15/35	13.8	25/25	12.3	30/30	12.8	35/35	10.5
20/15	15.0	-	-	-	-	-	-

s.e.d = 0.2887 replication = 2

d.f = 33 cv = 2.1%

Mean Germination Time

Results presented in Table 4 showed that there were significant differences ($p < 0.001$) between mean germination time (MGT) recorded at the various temperature regimes. The shortest mean germination time recorded included: 10.4 days at 25/30°C (100.0%); 11.2 days at 30/35°C (93.0%); 12.3 days at 25/20°C (96.5%); 12.4 days at 20/25° C(100.0%); 12.3 days at 25/25° C(87.0%); 12.8 days at 30/30°C (83.5%). The longest mean germination time recorded included: 17.4 days at 10/25°C (67.0% germination); 17.0 days at 10/35°C (47.0%); 16.5 days at 35/20°C (53.0%); 16.1 days at 25/10°C (70.0 %) and 15.8 days at 35/5°C (73.0%).

Time for First Germination

The time for first germination was significantly ($p < 0.001$) affected by the temperature regimes at which seeds were placed for germination on the thermogradient plate (Table 5). The earliest time for first germination was 8 days after seeds were placed for germination at temperature regimes 20/35°C, 30/30°C, 30/35°C and 35/35°C. The slowest time for first germination was 13 days after sowing at 20/15°C and 10/35°C; 13.5 days after sowing at 10/25°C and 35/20°C; 14 days after sowing at 25/10°C.

Seed Germination Velocity (T_{50})

The germination velocity or rate expressed as T_{50}, which is days to reach 50.0% of the final germination percentage was significantly ($p < 0.001$) affected by the temperature regimes at which seeds were placed for germination on the thermogradient plate (Table 6). The shortest time to attain T_{50} was 10 days at temperature regime 30/35°C with 93.0% germination. A T_{50} of 11.5 and 12 days attained at temperature regimes 25/20°C; 25/25°C; 15/25°C ; 20/25°C; 20/30°C and 25/30°C were also significantly lower than days to achieve T_{50} at the other temperature combinations. The longest time to achieve 50.0% cumulative germination was 20 days at 10/35°C (47.0%) and 18 days at 10/25°C (67.0%).

Cumulative Germination Percentage Curves

Cumulative germination percentage curves of *Khaya anthotheca* at selected temperature regimes are presented in figures 2a to 2e. At the alternating temperature of 25/20°C, the germination percentage increased until the 5th day. The largest germinability was observed during this period after which germination remained constant. At 30/35°C, germination percentage increased until the 11th day and remained constant afterwards. The largest germinability was observed between the 3rd and 5th day. At 5/30°C, maximum germination (gMax) was reached on the 13th day. The largest germinability was recorded between the 5th and the 7th day (Figure 2a).

At the alternating temperature of 10/30°C, germination percentage of the seed increased until the 7th day, it then remained constant till the 4th day and further increased slightly up to the 6th day and finally remained constant from then on. The largest germinability was registered between the 5th and 7th days Seeds incubated at the temperature regimes 30/20°C and 25/15°C had increased germination and attained maximum germination (gMax) on the 9th day. The largest germinability at these temperatures regimes occurred between the 5th and the 7th days (Figure 2b). Seeds subjected to the alternating temperature regimes of 25/30°C and 20/35°C continuously had increased germination percentages attaining maximum germination (gMax) at days 7 and 13 respectively. The largest germinability occurred between the 3rd - 5th day and the 5th – 7th day respectively for the two temperature regimes (Figure 2c).

At the temperature regimes of 20/25°C and 35/15°C, seed germination percentage increased continuously attaining the maximum germination on the 7th and 13th days respectively. The largest germinability was recorded between the 1st and 3rd days at 20/25°C. Seeds placed at 35/15°C had their largest germinability between the 5th and 9th day (Figure 2d)

Seeds incubated at 15/25°C gave increased germination percentage until the 9th day, remaining constant after this period. The largest germinability occurred between the 5th and 7th day. Seeds incubated at 15/30°C gave increased germination percentage until the 13th day and remained constant after this period. The largest germinability was registered between the 5th and the 7th day. At a constant temperature of 20/20°C, germination percentage increased up to the 9th day and remained constant afterwards. The largest germinability was registered between the 5th and the 7th day (Figure 2e).

Table 5: Time to first germination(days) of *Khaya anthotheca* seeds incubated at different temperature combinations on the 2-way thermogradient plate. Only temperature regimes where germination occurred are shown

Temp. (°C)	Time to 1st germination	Temp (°C)	Time to 1st germination	Temp. (°C)	Time to 1st germination	Temp. (°C)	Time to 1st germination
5/30	12.0	20/20	12.0	25/30	10.0	30/35	8.0
5/35	10.0	20/25	12.0	25/35	10.0	35/5	11.5
10/25	13.5	20/30	10.0	30/5	12.0	35/10	12.0
10/30	12.0	20/35	8.0	30/10	12.0	35/15	12.0
10/35	13.0	25/10	14.0	30/15	10.0	35/20	13.5
15/25	12.0	25/15	12.0	30/20	12.0	35/25	12.0
15/30	12.0	25/20	10.0	30/25	10.0	35/30	10.0
15/35	10.0	25/25	10.0	30/30	8.0	35/35	8.0
20/15	13.0	-		-		-	

s.e.d = 0.2132 replication = 2

d.f = 33 cv = 1.9%

Table 6: Time to 50 % cumulative germination (T_{50}) in days of *Khaya anthotheca* seeds incubated at different temperature combinations on the 2-way thermogradient plate. Only temperature regimes where germination occurred are shown

Temp (°C)	T_{50} (days)	Temp (°C)	T_{50} (days)	Temp. (°C)	T_{50} (days)	Temp. (°C)	T_{50} (days)
5/30	14.5	20/20	13.5	25/30	12.0	30/35	10.0
5/35	12.0	20/25	12.0	25/35	14.0	35/5	16.0
10/25	18.0	20/30	12.0	30/5	14.0	35/10	14.0
10/30	13.5	20/35	14.0	30/10	14.0	35/15	16.0
10/35	20.0	25/10	15.0	30/15	14.0	35/20	16.0
15/25	12.0	25/15	14.0	30/20	13.5	35/25	16.0
15/30	13.5	25/20	11.5	30/25	12.0	35/30	15.0
15/35	13.5	25/25	11.5	30/30	14.0	35/35	15.0
20/15	13.0	-		-		-	

s .e.d = 0.4924 replication = 2

d.f = 33 cv = 3.5%

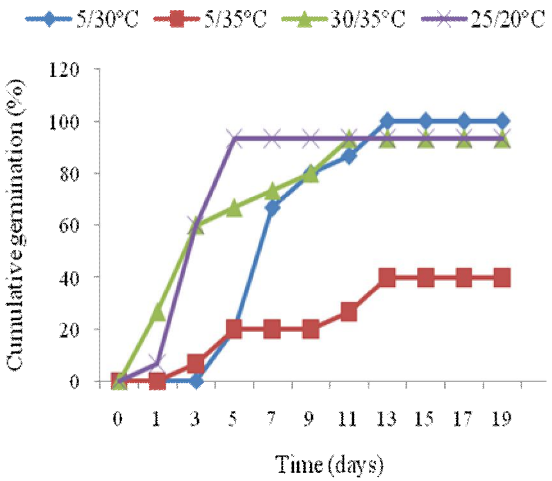

Figure 2a: Cumulative seed germination (%) of *Khaya anthotheca* at four temperature combinations on the thermogradient plate

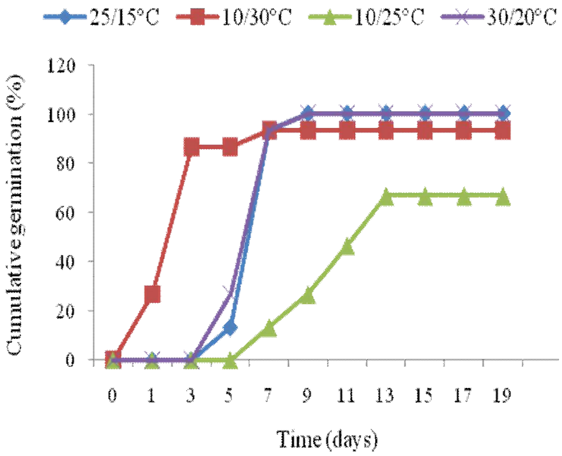

Figure 2b: Cumulative seed germination (%) of *Khaya anthotheca* at four temperature combinations on the thermogradient plate

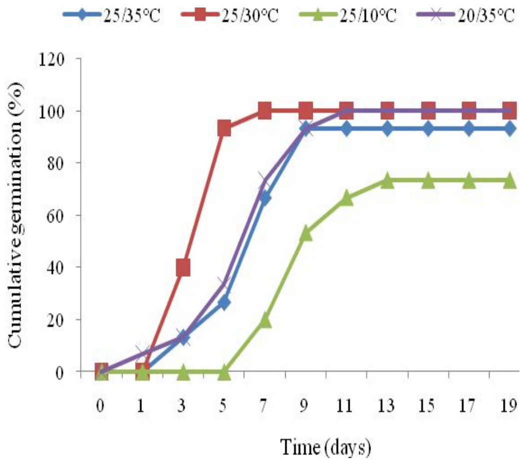

Figure 2c: Cumulative seed germination (%) of *Khaya anthotheca* at four temperature combinations on the thermogradient plate

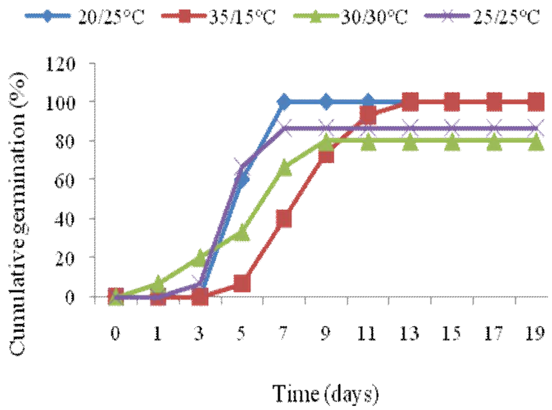

Figure 2d: Cumulative seed germination (%) of *Khaya anthotheca* at four temperature combinations on the thermogradient plate

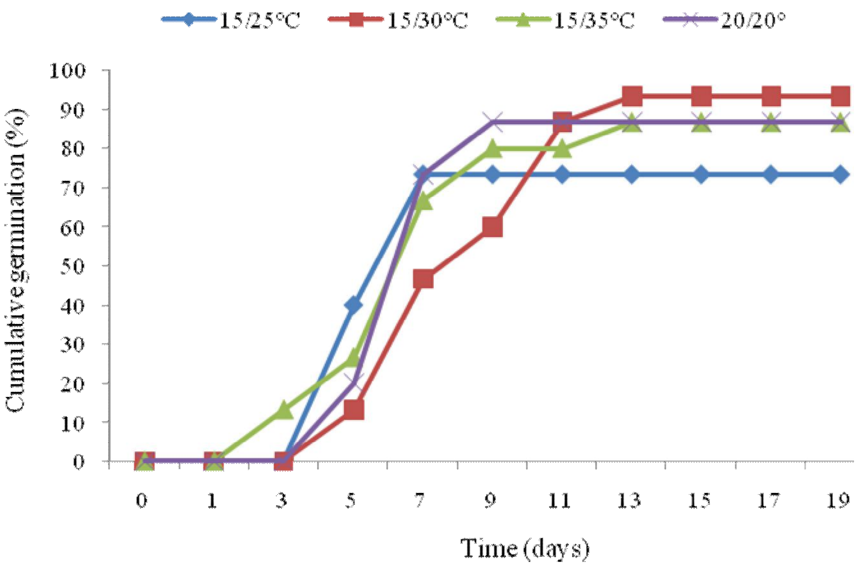

Figure 2e: Cumulative seed germination (%) of *Khaya anthotheca* at four temperature combinations on the thermogradient plate

DISCUSSIONS

The relatively low seed moisture content and equilibrium relative humidity (eRH) of *Khaya anthotheca* on receipt at the laboratory (Table 1) indicated that the species had gone through the process of maturation drying like all orthodox seeds as reported by Song *et al.* (2003). In spite of this there was the need to further dry the seeds to reduce seed moisture and for that matter seed eRH form the ‘‘damp’’ status to the ‘‘dry’’ status (MSBP, 2005) to enhance it storage before the germination study was conducted.

The observation that germination of *Khaya anthotheca* occurred in thirty three (33) out of the sixty four (64) temperature combinations made possible by the thermogradient plate is an indication that the species has the ability to germinate over a wide range of alternating and constant temperature regimes.

Khaya anthotheca seeds will not germinate at regimes of low alternating temperatures as well as regimes of low constant temperatures. For example zero germinations were recorded at the following alternating temperature regimes: 5/10°C; 5/15°C; 5/20°C; 10/5°C; 10/15°C; 15/5°C; 15/10°C; and even at 15/20°C; 15/25°C; 20/5°C and 20/10°C. Zero germination was also recorded at constant temperature regimes of 5/5°C; 10/10°C and 15/15°C. This observation affirms the fact that the optimal germination temperature of tropical species is generally relatively high (Daws *et al*. 2002). According to Asomaning (2009), seeds of the species germinated equally well at constant temperatures between 20-35°C, germination percentages of about 85% were recorded at each of these temperatures without any chemical pre-treatment. Germination of *K. anthotheca* was greatly enhanced by alternating temperatures compared to germination percentages recorded at constant temperatures even though the species is not known to have any dormancy problem. Germination at alternating temperatures such as 5/30°C; 10/30°C; 15/30°C; 20/25°C; 20/35°C; 25/15°C; 25/20°C; 25/30°C; 30/5°C; 30/10°C; 30/15°C; 35/10°C; and 35/15°C among others gave between 90 and 100 % germination. Between each of these alternating temperatures, amplitude of change between day and night was not more than 25°C. It is evident that amplitude of change between day and night of 20 to 25°C and not more was good for the germination of the seed. Experiments with 2-way thermogradient plate revealed that germination of many temperate species occurred at a wide range of temperature regimes, and that amplitude of change between day and night of 10 to 12°C might have been more important than the cardinal points (Bonner, 1983).

The observation that temperature combinations which had the highest final germination percentages achieved this with minimum mean germination time compared with temperature combinations which recorded low final germination percentages (Tables 2 and 3) is in agreement with the findings of Kochankov *et al*. (1998) who reported that in *Echinacea purpurea* (L.), the highest germination percentage was recorded for seeds with the shortest mean germination time. Silviera *et al*. (2005) and Asomaning (2009) also reported of a similar observation in *Calliandra fasciculata* (Benth.) and *Terminalia superba* respectively.

Temperature regimes which recorded faster time to first germination invariably also recorded low mean germination time, indicating rapid germination and high final germination percentage (Tables 4 and 5). This mean that when seeds have been placed at their optimal temperature regimes they will soon go through the germination process compared to when they have been place at adverse temperature regimes. Time to 50% cumulative germination (T_{50}) at temperature regimes which recorded high final germination percentages was also considerably lower compared to regimes which recorded lower final germination percentages (Table 3 and 6).

The steepness of the cumulative germination percentage curves of *Khaya anthotheca* seeds incubated at the various temperature regimes showed differences in increases in germination percentages over time, periods of largest seed germinability, continuity in seed germination and periods at which germination remained constant and when maximum germination was attained (Figures 2a to 2e). The steepness of the curves revealed how fast or otherwise the biological relevant parameters namely: the final germination percentage, the mean germination time, rate of germination and time to first germination which informed the dynamics of the germination process measured in this study were attained at these incubation temperatures. Labouriau (1978) and Kocabas *et al*. (1999) reported that temperature affects the germination capacity (germinability), the germination rate, and the distribution of the relative frequency of germination along the incubation time. In general, the cumulative germination of *Khaya anthotheca* at all the temperatures over time appeared as S-Shaped curves (Figures 2a to 2e) resembling typical cumulative germination of a population of seeds over time as reported by Shafii and Price (2001).

CONCLUSION

Temperature regimes had significant effect on final germination percentage, mean germination time, time for first germination and rate of germination of K. *anthotheca* seeds. Alternating temperatures improved overall germination. The best germination at a constant temperature was at 20/20°C and 30/30°C. The best temperature regimes for seed germination at alternating temperatures were at 5/30°C, 10/30°C,15/30°C, 20/25°C, 20/35°C, 25/15°C, 25/20°C, 25/30°C, 30/20°C, 35/10°C, and 35/15°C

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