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Patula pine (*Pinus patula*) cones opening under different treatments for rapid seed extraction in Londiani, Kenya

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Seed extraction from pines is challenging to the forestry sector globally. This is usually contributed by the pine cone anatomy that opens through a function of temperature and humidity which varies widely in the pine growing regions of the world and the *Pinus* species as observed in previous works done on *Pinus roxburghii*, *Pinus halepensis, Pinus wallichiana*, *Pinus pinaster, Pinus radiata*, and *Pinus sylvestris*. This study sought to reduce the extraction time and improve the extraction efficiency of *Pinus patula* seed in Kenya. The experimental design used was two factorial design with replicates of twenty cones randomly picked per treatment. Data analysis was analyzed through ANOVA with a P-value of 0.05. We present evidence that soaking does not influence the opening of cones and seed yield for the optimum temperature which the study determined to be 65°C. We also present evidence that, for rapid seed extraction, the temperature 65°C with an exposure period of between 4 and 24 h is significantly effective. This study presents a new understanding of rapid seed extraction, which contributes to one of the Kenya Forestry Research Institute's strategic objectives of generating technologies for enhanced production of superior germplasm for priority tree species.

Key words: Soaking effects, temperature treatments, seed yield.

INTRODUCTION

Seed extraction from pines globally is challenging to the forestry sector (Bhat et al., 2017; Reyes and Casal, 2001) with most countries using the conventional methods for seed extraction from cones, which entails drying in beds to facilitate seed release. This weather-dependent process is particularly slower and less efficient in moist; cool temperate climates as an increase in the atmospheric humidity may cause a reclosing of the cones (Willan, 1984). In moist conditions, this method is not very reliable. However, it is the most economical, convenient, and effective method of seed extraction for many cone-bearing species (Bhat et al., 2017; Ghildiyal et al., 2008). This is due to the fact that the process in most countries is based on natural sun drying in canvas or drying beds to facilitate the release of seeds (Bhat et al., 2017; Wyse et al., 2019).

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The major preferred species used in commercial plantation forestry are Eucalypts, Cypress, and Pines (Essl et al., 2011; Ngugi et al., 2000). Sustainable plantation forest production demands a continuous supply of high-quality seeds for the production of seedlings in the nursery or for direct sowing (Ridder, 2007). Previous studies have reported that pine cones open to release seed through a combination of factors (temperature and humidity) which varies widely in the pine growing regions of the world (Aniszewska et al., 2020b; Essl et al., 2011; Wyse et al., 2019). Another factor affecting the pine cone opening is the presence of resin with serotinous cones occurring in many species (Wyse et al., 2019). In these species, cone scales remain closed due to sealing with resin, which requires high temperatures for resins to melt and cones to open (Perry and Lotan, 1977; Wyse et al., 2019).

Patula pine (*Pinus patula*) is a straight bole tree with its origin from Mexico (Perry, 1992). It is has a wide distribution range including Southern and East Africa where it is commercially planted (Dvorak et al., 2012; Essl et al., 2011). In Kenya, *P. patula* represents 27% of plantation species among others such as *Cupressus lusitanica* and Eucalyptus species (Ngugi et al., 2000). It has been grown for industrial production for pulpwood and sawn wood which therefore exerts a high demand for seed extraction. There are scanty works of literature on *Pinus patula* seed extraction efficiency with most studies focusing on other pine species (Ayari and Khouja, 2014; Bilir et al., 2008; Reyes and Casal, 2001; Singh et al., 2017; Wyse et al., 2019). Earlier work by Calvo and Nu (2000) has also revealed that the primary factor for seed release in Aleppo and Scots pines is the degree of cone opening, with few studies on other pine species. Many factors have been attributed to poor cone opening such as early harvesting, fungal attacks, insect damage and case hardening during storage with little data on soaking and temperature effects which mimic field conditions (Ayari et al., 2011, 2012; Ayari and Khouja, 2014; Bramlett, 1977). The lack of understanding of these effects has led to a combined reduction of available *P. patula* seeds.

The case of Kenya is marked by *P. patula* being one of the commercial species in the country. Kenya Forestry Research Institute (KEFRI), Londiani centre is the source of pine seed for the country (Albrecht, 1993). The major challenge faced by KEFRI is the prolonged extraction periods, thereby delaying the availability of the seed. Thus, there is need to reduce the extraction time and improve extraction yield in terms of quantity and quality. This paper sought to determine the effect of varying temperatures on *P. patula* cone opening for seed extraction, with specific objectives being: (i) to determine effect of soaking on cone opening, (ii) to determine the effect of various temperatures on percent opening of cones and (iii) to assess the combined effects of soaking and temperature treatments on cone opening and seed yield.

MATERIALS AND METHODS

Mature *P. patula* cones samples were randomly collected from a wider general collection batch whose sources were an assortment of seed stands and orchards. The cones were closed at the time of collection. The cones were packed in gunny bags and then brought to the KEFRI Rift Valley Eco-Region Research Programme – laboratory in Londiani, positioned between 35.607270°E longitude and 0.155520°S latitude.

Londiani area is cool and moist in most part of the year and is at an elevation of 2,308 masl with an average temperature of 15.7°C. The average temperatures around the forest reserve are between a minimum of 8.6°C and a maximum of 23.31°C. The area has two rainy seasons, long rains occurring in the months of March to May with an average rainfall of 750 mm for the three months, and short rains in October to December with average rainfall for the three months of 423 mm. The driest months are January to February and August to September. This study was conducted in March 2020, which is the peak cone production season for Patula pine (Albrecht, 1993).

Experimental design

The cones collected were assessed for maturity, defects (already opened at least once and closed; immature and pests damage). This was a factorial experimental design with temperature and soaking conditions as factors and exposure periods as levels. Twenty replicates were used for each treatment. Cones were exposed to soaking in hot and cold water for varying periods (10 min and 24 h) to simulate the varying humidity effects (Aniszewska, 2013). The design is as represented in Table 1. Cones in each treatment group were labeled and measured for length (cm), diameter (cm), and weight (g). Cones were then soaked in hot (100°C) and room temperature (25°C) water for different durations: 10 min and 24 h. The cones were placed in labeled glass Petri dishes with sufficient space to prevent contamination of seeds from one cone to another.

The cones were subjected to artificial heating for seed extraction at eight temperature conditions: 30, 40, 50, 65, 70, 75 and 85°C (Schmidt, 2000) and DB (drying bed conditions to simulate the current practice for seed extraction; 44.8±6.00°C) at three exposure times (4, 24, and 48 h) together with the control (no soaking). The soaked and not soaked cones were then subjected to oven (YAMATO DS411) temperatures of 30, 40, 50, 65, 70, 75 and 85°C (Schmidt, 2000) and DB (natural sun drying conditions in a drying bed). The extraction exposure times varied for set temperatures: 48 h for lower temperatures (30 and 40°C and DB); 24 h for 50 and 65°C: and 4 h for higher temperature (70, 75 and 85°C). This was modified according to a previous study by Aniszewska et al. (2019), which showed that longer exposure durations at higher temperatures reduced seed vigor. Petri dishes with cones were removed immediately at the end of the exposure times from the oven and drying bed. Seeds were then extracted from cones in each Petri dish by tapping gently for 15 times on a flat wooden bench and counted. Length (cm) of the part of the cone that had opened was measured as well as cone weight (g).

Data analysis

The data were tabulated in a data-sheet in MS excel and analyzed for: effect of soaking on percent opening and seed yield; effects of temperature on percent opening and seed yield and combined

Table 1. Experimental design.

S/N	Treatment	Sample size
1	No soaking, drying bed, 48 h	20
2	No soaking, oven 30ºC, 48 h	20
3	No soaking, oven 40ºC, 48 h	20
4	No soaking, oven 50ºC, 24 h	20
5	No soaking, oven 65ºC, 24 h	40
6	No soaking, oven 70ºC, 4 h	20
7	No soaking, oven 75ºC, 4 h	20
8	No soaking, oven 85ºC, 4 h	20
9	Soaking in cold water for 10 min, Drying bed, 48 h	20
10	Soaking in cold water for 10 min, oven 30ºC, 48 h	20
11	Soaking in cold water for 10 min, oven 40ºC, 48 h.	20
12	Soaking in cold water for 10 min, oven 50ºC, 24 h	20
13	Soaking in cold water for 10 min, oven 65ºC, 24 h	20
14	Soaking in cold water for 24 h, oven 65ºC, 24 h	20
15	Soaking in cold water for 24 h, oven 70ºC, 4 h	20
16	Soaking in cold water for 24 h, oven 75ºC, 4 h	20
17	Soaking in cold water for 24 h, oven 85ºC, 4 h	20
18	Soaking in hot water 24 h oven 65º 24 h	20
19	Soaking in hot water 24 h, oven 70º 4 h	20
20	Soaking in hot water 24 h, oven 75º 4 h.	20
21	Soaking in hot water 24 h, oven 85º 4 h.	20

Figure 1. *P. patula* cones showing stages of cone opening.

effects of soaking and temperature on percent opening and seed yield done with RStudio Version 1.2.1335. Post hoc analysis (Tukey's HSD) was used to determine difference in means (95% CI) in the two-way ANOVA with soaking, the exposure time for soaking, temperatures and exposure times for temperatures as factors and percent opening and seed yield as variables.

RESULTS AND DISCUSSION

The conventional practice in Kenya for extracting seeds from cones of *P. patula* consists of drying cones in the sun. The process (DB) takes about two weeks for complete seed extraction. This paper's results from the DB conditions were attributed to the diurnal temperature variation of 44.8±6.00°C.

Cones were observed to open from the widest part of the cone to the top section (Figure 1). This was in agreement with previous studies on other pine species that show, longer scales were located in the middle to the top part of the cone (Aniszewska, 2010). These studies

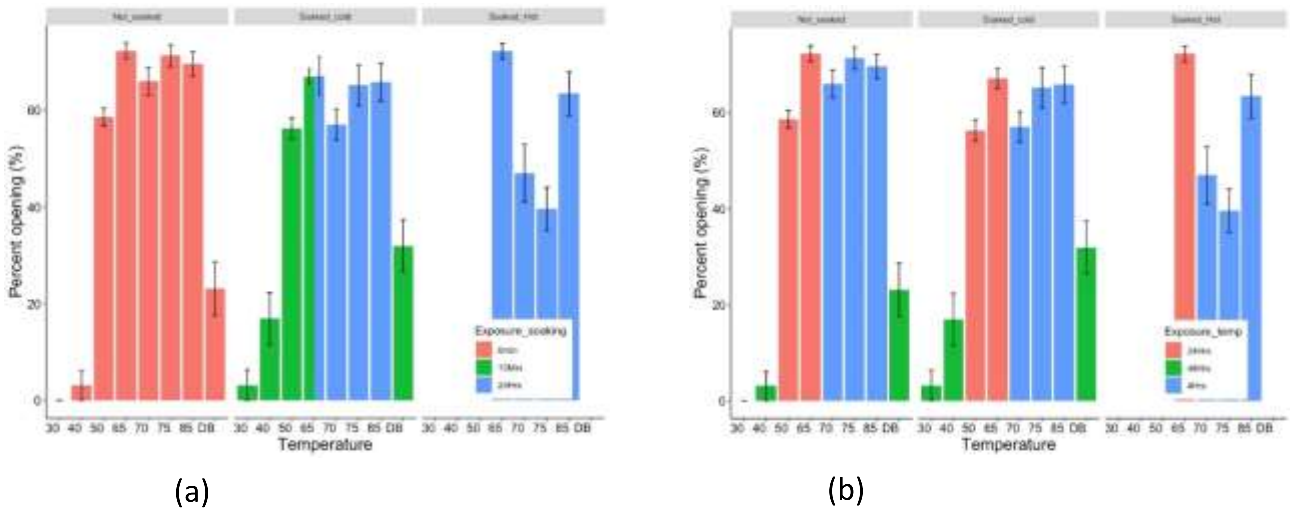


Figure 2. *P. patula* cones percent opening based on exposure times to soaking in hot and cold water (a) and percent opening based on oven exposure at different temperature regimes (b).

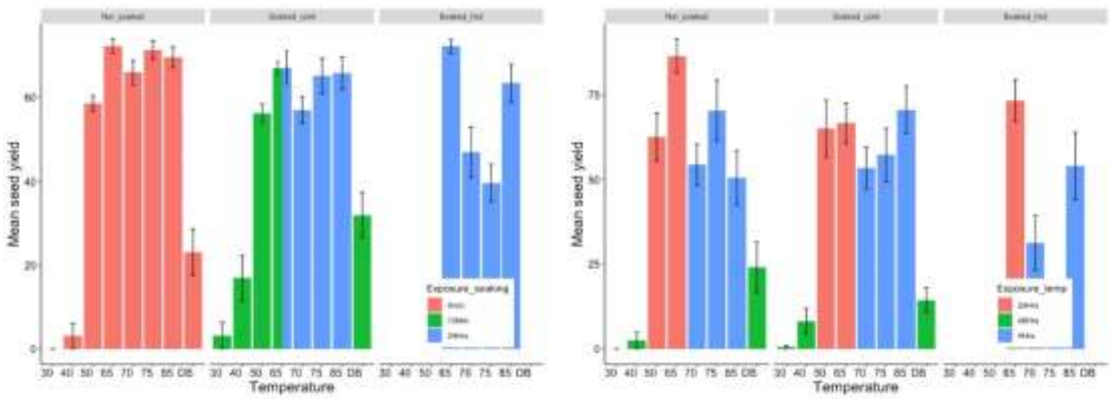
noted that the longer scales had higher deflection compared to scales at the base (short), which attributes to the base part of the cone not to open (Wyse et al., 2019; Aniszewska, 2010; Reyes and Casal, 2001).

The results of this study showed that soaking (50.2±1.69) or not soaking (48.5±2.36) of cones did not have a significant effect ($p>0.05$) on the percent opening of the cones from the temperature treatments. This study observed that when soaking temperature treatments are isolated (soaking in room temperature water and soaking in hot water) for their effects on percent opening of the cone, and there were significant differences ($p<0.05$) (Figure 2b). Soaking in hot water significantly influenced the cone opening with the highest mean percent opening (55.5±2.62%) ($p<0.05$). This study observed that 65°C had highest percent opening of cones after soaking in hot water (72.2 ±1.62%), soaking in cold water (67.1±2.11%) and for cones subjected to no soaking (75.0±3.01%) (Figure 2b). Soaking of cones in either hot or cold water increases the moisture content of the cone, which impedes the quick opening of the cones. This finding agrees with earlier work by Wyse et al. (2019) and Ghildiyal et al. (2008) and who reported that moisture content is a factor for cone opening.

The variations in cone opening based on different temperatures were noted to be similar to previous studies on other pine species (Wyse et al., 2019; Bilir et al., 2008; Iwaizumi et al., 2008). Within our study, cones opening subject to the temperatures of 30 and 40°C were observed to be few, with many remaining closed. There are studies that suggested more reasons for variation in cone opening such as cone morphology, degrees of asymmetry, differences in scale tension, the effect of environment on bonding strength and genetic differences in bonding agent (Aniszewska et al., 2020a; Perry and Lotan, 1977). The reasons need further investigation focusing on *P. patula* cones, though, this study agrees with previous findings on other pines that also observed temperature being a key factor for cone opening (Bhat et al., 2017; Ghildiyal et al., 2008). During the study, it was observed that at temperatures from 50 to 85°C the number of cones opening ranged from 95 to 100% per temperature treatment. The number of cones opening decreased with reducing temperature: DB (63%), 40°C (20%), and 30°C (2.5%) under 48 h exposure. This finding suggests the need for longer exposure for these temperatures, which agrees with observations from other studies (Bhat et al., 2017; Singh et al., 2017). At exposures of 4 and 24 h, 65°C was the most effective temperature for cone opening, with the results having no significant differences ($p<0.05$) in percent opening between 4 and 24 h (Figure 2b).

The least mean seed yield (43.3±4.50) was observed from cones soaked in hot water. This implies that soaking in hot water causes additional moisture to be absorbed by the cones thereby requiring a higher exposure for the cones to lose the additional moisture. Earlier studies show high temperature melts resin in the cone (Perry and Lotan, 1977; Wyse et al., 2019), thus with melted resin, the cone imbibes more moisture hence not suitable for rapid seed extraction (Figure 3a and b). Percentage opening of pine cones was observed to have a significant influence on the seed release for all the temperature treatments except for the 50°C treatment ($p>0.05$) (Figure 3a and b). An analysis by temperature and treatment from this study observed cones not exposed to the soaking treatment yielded the highest number of seeds (86.5±4.94) at temperature 65°C (Figure 3a, b). Further analysis by temperature treatment still observed 65°C having a mean seed yield of 75.9±3.41.

The overall combined effects of soaking water temperatures, soaking exposure, oven temperatures, and



(a) (b)

Figure 3. *P. patula* cones mean cumulative seed yield based on exposure times to soaking in hot and cold water (a) and mean cumulative seed yield based on oven exposure at different temperature regimes (b).

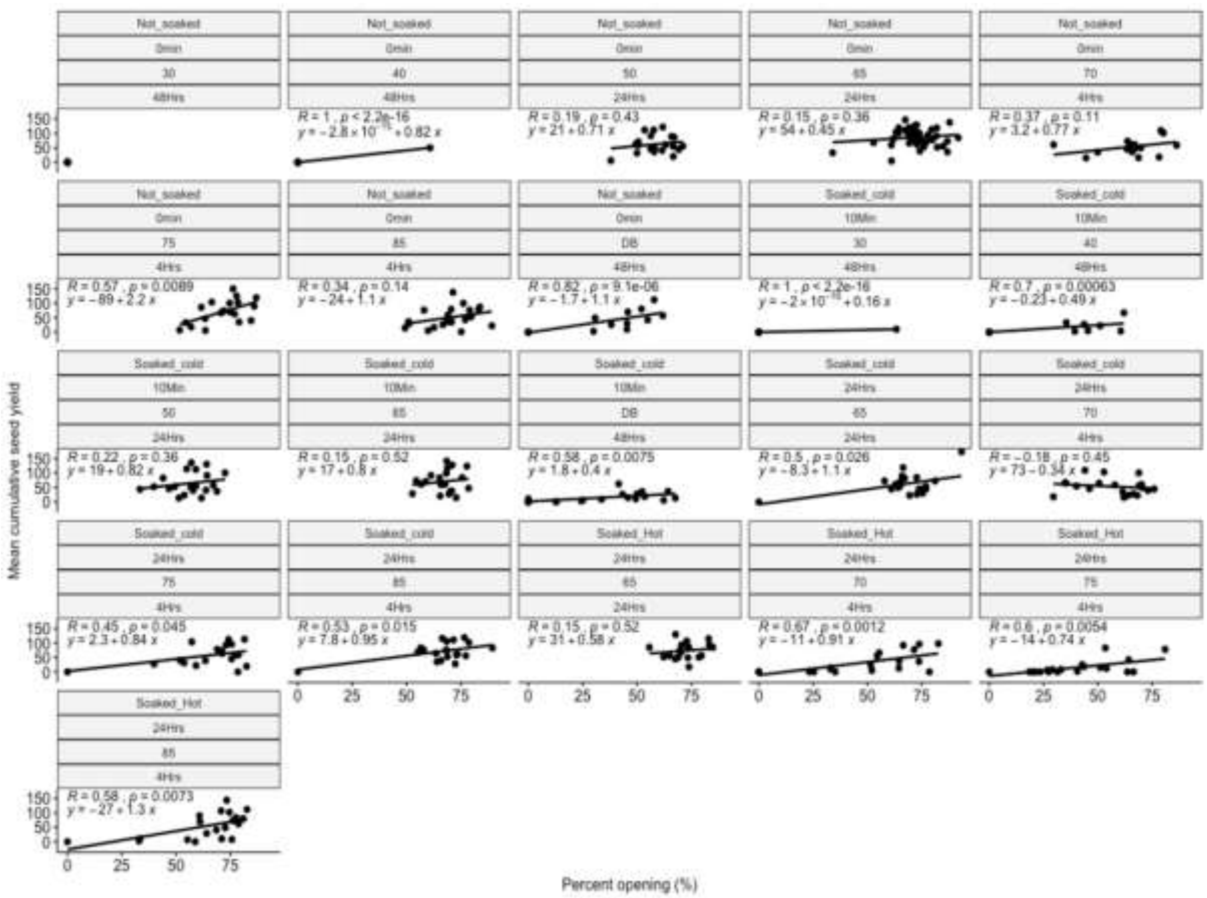


Figure 4. Correlations of combined effects of soaking and temperature exposure on *P. patula* cones percent opening and seed yield.

oven exposure time established that 65°C is the best performing temperature for rapid seed extraction. The results showed a positive correlation between percent opening and seed yield under the different treatments except for 24 h soaking in cold water and oven exposure of 4 h at 70°C (Figure 4). Higher temperatures (>75°C) are known to be less suitable due to the possibility of greater damage to seeds when exposed to this temperature (Calvo and Nu, 2000). This identifies the need to study seed viability when the extraction is carried out at higher temperatures.

Artificial heating for drying of cones at controlled temperatures requires expensive equipment and installations, which are seasonally used. This makes their unit cost exceptionally high. A very careful appraisal must, therefore, be made of the capital cost before establishing a large permanent kiln (FAO, 1985). There is, however, the scope for adapting the best procedure found in this study to many nursery conditions. There was a significant increase in the number of seeds extraction on cumulative percent basis as the temperature was increased from 50 to 65°C. The opening mechanism of *Pinus patula* cones scales under oven and drying beds conditions is related to temperature and moisture differences. The present investigation on *P. patula* indicated that seed extraction temperatures exert a significant influence on the number of seeds extracted on a cumulative basis. The authors, therefore, recommend the use of artificial heating of cones at 65°C for 4 to 24 h for the rapid extraction of *P. patula* seeds.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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