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Full length Research paper

Effects of seed pre-treatment and media on seed germination and seedling growth of Iroko, *Milicia excelsa* (We/w.) C.C. Berg moraceae, Syn. Chlorophora excelsa

Nzekwe, U.¹, Ubani, C. S.²* and Ajuziogu, G. C.

¹Department of Botany, University of Nigeria, Nsukka, Enugu State, Nigeria. ²Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria.

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The effects of seeds pre-treatment and media, on germination of the seeds and seedling development of Iroko, *Milicia excelsa*, an important but threatened timber yielding species, were assessed. *M. excelsa* seeds extracted from the fruits that dropped from the trees were subjected to various pretreatments [(washing in tap water, (SB₁), washing in water and soaking for 15 min in hot water, 50° and 60° C, respectively (SB₃ and SB₄), washing in water, soaking in hot water 50 and 60° C, respectively, then left till hot water cooled (SB₄ and SB₆) and SB₁, is the control,] before sowing in 6 different media; (garden soil (GS), sawdust (SD), 1:1 mixtute of GS + SD, GS + PD (Poultry droppings), SD + PD and 1:2:1 mixture of GS + PD + SD). Pre-treated seeds; SB₂, SB₃, SB₅ germinated within 2 weeks after sowing, the control, SB₁ germinated within 3weeks while SB₄ and SB₆ failed to germinate. GS + PD + SD medium consistently influenced the highest percentage germination responses. Within the pre-treated seeds SB₃ gave the highest percentage germination (92%) and the control, the least 63%, while SB₄ and SB₆ failed to germinate. Variations shown by mean values of some seedling aerial growth parameters showed that the best values were consistently obtained with seedling grown in GS + PD + SD medium. The study has shown that *M. excelsa* can be propagated by the seeds.

Key words: Milicia excelsa, seed pretreatement media.

INTRODUCTION

Prior to the exploration of petroleum, timber in the form of log, sawnwood and roundwood constituted the main bulk of Nigeria's export commodies (Enabor, 1976; Nwoboshi, 1976). Redhead (1971) identified 560 plant species in Nigeria's forest capable of yielding timber. Nwoboshi (1976) observed that 60 out of the 560 plant timber species identified by Redhead (1971) can yield timber of commercial value. Currently, rapid economic development and large scale deforestation have ostensibly increased timber consumption in Nigeria, thus resulting in the diminishing trend in the supply of high grade timber in Nigeria (Adeyoju, 1971; Enabor, 1976). Due to superior position of timber demand over supply, the existing plantations may not meet future demand for wood (FAO, 1970; Odeyinde, 1980). Earlier, Redhead (1971) listed *Chlorophora excelsa, Khaya ivorensis, Nauclea didirechi, Gmelina arborea, Terminalia ivorensis* and a host of other plant species as the major economic timber species in Nigeria. Since then, except *C. excelsa, M. excelsa,* (Iroko) and most of the identified timber

*Corresponding author. E-mail: chike.ubani@unn.edu.ng.

species of economic importance have been established in plantations and have also received research attention.

Iroko, *M. excelsa,* (We/w) C. C. Berg belongs to the family Moraceae (Keay et al., 1964). The species is synonymous with *Maclura excelsa* (We/w) Bureau and *Chlorophora excelsa* (We/w). Beuth. The species is variously recognized in tropical Africa: Iroko, rock elm, African teak, and African oak. The species has a close relative, *Milicia regia* (A.Chev) C. C. Berg. The wood of the two relatives are not easily distinguishable in timber trade under the trade name, Iroko.

M. excelsa is reported to have several origins; Ethiopia, through Guinea Bissaua to other tropical African regions. The species is introduced into India and the United states (Ofori, 1982). The wood of the species is highly sought after globally in commercial timber trade, due to its excellent characteristics (quality), hence the wood is used in various productions; construction works, ship-building, marine carpentry, railway sleepers, sluice gates, furniture, cabinet works, pulp and paper and numerous other industrial productions (Ofori, 1982). M. excelsa wood has; a density of 550 to 750 kg/m² at 12% moisture content, shrinkage rates from green to oven dry are; 1.7 to 4.1 (-56%) radial and 2.4 to 6.3 (-9.3%) tangential (Ofori, 1982). The species is highly valued not only for its large volume of wood content but also for its peculiar durability in service. Findlay (1975) and Odeyinde (1980) reported that the untreated wood of the species has a service life expectance of about 25 to 30 years. Ofori (1982) reported that the wood dries well in open air and kilns with little degrade.

In Africa, particularly Nigeria, the Igbos consider M. excelsa a sacred tree especially when the tree grows within a village square, such tree is often protected. The species is alleged to have long gestation period, hence, the adage of the Igbos of Nigeria, "He that plants Iroko never lives to harvest it". The tree produces clusters of finger-like fruits between November and April. The fruit contains hundreds of very tiny seeds that are surrounded by sticky oil substance. Mature fruits are food for Bats, Chiroptera spp, the species main dispersal agent. The bats after feeding on the fruits pass out the seeds alongside their fecal matter and the seeds later germinate under favourable conditions (Hills, 1952). The tree is deciduous; up to 50 m tall, bole is straight, cylindrical and grows branchless at about 20 to 30 m height, with diameter, of 2.5 m to 3 m. Trees surface roots are prominent, long and big (Richardson, 1976; Ofori, 1982).

Apart from timber, the species has been reported to be of use in traditional healthcare delivery (Anon, 2005). Decoction of the root is used in treating female infertility, while decoction of the root and stem bark is used in cuing ailments such as; cough, asthma, heart problems, lumbago, and numerous other diseases (Ofori, 1982; Anon, 2005).

Despite the enormous potential of *M. excelsa,* and its contributions to human socio-economy, the species resources are still obtained from the wild, because the

species has not been included in the various agro forestry/conservation programmers' of many countries especially in Africa. Currently, the species is endangered due to over exploitation, deforestation and numerous human activities. Myers (1989) reported that Nigeria is among the twelve nations whose deforestation rate accounts for over 80% of the global total. The rapial disappearance of the forest and diminishing supply of high quality timber like M. excelsa have resulted in the use of low grade and immature timber of other plant species. Detailed information on the propagation of the species (sexually and vegetatively) appears scanty. Ofori (1982) listed *M. excelsa* among the plant species that can be propagated vegetatively by grafting and air layering, but did not give details. Ofori et al. (1996) reported successful rooting of the leafy stem cuttings of the species. The authors further pointed out that stem cuttings could be rooted with the application of 0.2% indole-3-butvric acid (IBA) on the stem cutting with 40cm² leaf areas and planted in composted sawdust of 24% water holdings capacity.

Our main objectives are: assessment of the effects of seed pre-treatment and media on the germination of the seeds of *M.excelsa*. The results of the study if successful could contribute in the conservation of the species that is at present threatened.

MATERIALS AND METHODS

Ripe fruits that dropped from the trees in the premises of the Faculty of the Biological Sciences, University of Nigeria, Nsukka (UNN), Enugu state, long 6°42′ and 6° 49′ E and Lat. 5°.56′ U and 6° 03′ N were collected in February 2009. The collection area, UNN, has an altitude of 120 m above sea level with an average rainfall of about 1,200 mm per annum, (March to October), distributed over period of 7 months, with the most humid peak in June to July and a short dry spell in August. The location, UNN lies within the derived savanna woodland zone with mean daily temperature of about 29° to 31°C through the year.

The fruit were packaged in large labeled brown envelopes, while the twigs were pressed following herbarium methods (Keay, 1989). The materials were identified and confirmed in the herbarium of the Department of Botany, UNN, following the process described by Hutchinson and Dalziel 1963). Prior to use, the fruits were stored in a dessicator for 4 days to soften, after which the seeds were extracted mechanically by pressing the fruits between the fingers. The bulk of extracted seeds were subjected to viability test (Bcwley and Black, 1985). Seeds that floated were decanted being considered non-viable, while seeds that sank were air dried for 30 min before use. 1440 seeds were randomly selected from the presumed viable seed bulk and stored in a dessicator for 2 days at room temperature before use.

The seeds were further divided into six batches of 240 seeds each for various pre-treatments (Table 1). Un-pretreated seeds were included as the control and for comparison. The seeds were sown in 6 different media formulated as shown in Table 2. Acid scarification was not included in the pre-treatment of the seeds because the method adopted in this study was aimed at nursery men and foresters adoption level.

Prior to sowing, samples of each medium were taken for physicochemical analysis, with emphasis on their water holding capacity and nitrogen content. Poultry droppings incorporated in the

Seed batch (SB)	Description of pre-treatment
SB1	Control, sown wet
SB ₂	Washed in tap water, air-dried
SB ₃	Washed in tap water, then soaked for 15 min in hot water, 50°C
SB ₄	Washed in tap water, soaked in hot water 50°C and remained till hot water cooled.
SB ₅	Washed in tap water, then soaked for 15 min in hot water, 60°C
SB ₆	Washed in tap water, then soaked in hot water 60°C and remained till hot water cooled.

Table 1. Pre-treatment of *Meletia excelsa* seeds before sowing.

Six different media assessed for their effects on seed germination are listed in Table 2.

Media symbol	Description of media			
GS	Garden soil, obtained around the Botanic Garden, Department of Botany, U.N.N.			
SD	Sawdust of a soft wood species, <i>Ceiba pentandra,</i> collected from a sawmill at Nsukka Urban timber market.			
GS + SD	1:1 mixture (50 kg each) of garden soil and sawdust.			
GS + PD	1:1 mixture (50 kg each) of garden soil and poultry droppings.			
SD + PD	1:1 mixture (50 kg each) of sawdust and poultry dropping			
GS + PD + SD	1:2:1 mixture (50 kg each of GS and SD), and 100 kg of poultry droppings.			

Table 3. Some physicochemical properties of the nursery media.

Media	Water holding capacity (%)	Total N (%)	
GS	36 ^{cd}	0.25 ^c	
SD	15 ^d	0.02 ^d	
GS + SD	62 ^{ab}	0.26 ^c	
GS + PD	54 ^{bc} 76 ^{ab}	0.31 ^{ab}	
SD + PD	76 ^{ab}	0.30 ^b	
GS + PD + SD	84 ^a	0. 44 ^a	
X	53	0.26	
SE	22.26	0.13	

Values with the same alphabet (s) along the vertical column are not significantly different (P = 0.05).

media was composted for 4 weeks before use. Large size poly-pots perforated at the sides and base were used as potting containers. 240 poly-pots were filled with each of the 6 media. Each treatment was replicated 4 times and each replication was sown up with 10 seeds at the rate of 5 seeds per poly-pot and at the depth of 1.5 to 2.0 cm. Two poly-pots gathered together constituted a replication.

The experiment, a completely randomized design (CRD) was displayed on an elevated concrete platform in the Botanic Garden of the Department of Botany, UNN and lasted for 8 months (February to September, 2009). The Poly-pots were watered daily and weeds were hand picked. Seeds were considered germinated when seedlings emerged 1.5 to 2 cm above the medium. As from the 4th week after sowing when there were no more seedling emergence, seedlings were tinned to 2 per poly-pot and allowed to grow for 8 months after sowing, after which the study was terminated.

Data obtained from physicochemical properties (Table 3), percentage seed germination (Table 4) and some seedling growth

parameters (Table 5) were analyzed following CRD procedure (GENSTAT, 2003). Mean separation to detect the effects of seed pre-treatment and media on seed germination was by least significant difference (LSD) at 5% probability level.

RESULTS

Data on some physicochemical properties of the 6 nursery media showed variations (Table 3) in that 1:2:1 mixture of GS + PD + SD medium consistently had the highest percentages of water holding capacity and nitrogen, respectively. However its percentage water holding capacity did not differ significantly from those of GS + PD and SD + PD, while its percentage nitrogen also did not differ significantly from that of GS+PD medium.

Seed batch -	Germination in various media (%)					
	GS	SD	GS + SD	GS + PD	SD + PD	GS + PD + SD
SB ₁	30 ^d	35 [°]	58 [°]	57 ^c	46 ^c	63 ^c
SB ₂	52 ^{ab}	60 ^{ab}	68 ^{ab}	67 ^{ab}	65 ^{ab}	86 ^a
SB ₃	58 ^a	64 ^a	72 ^a	69 ^a	70 ^a	92 ^a
SB ₄	-	-	-	-	-	-
SB₅	47 ^b	54 ^{ab}	63 ^{bc}	58 [°]	56 ^b	74 ^b
SBc	-	-	-	-	-	-
x	46.75	53.25	65.25	62.75	59.25	78.75
SE	10.42	11.12	5.26	5.31	9.15	11.17
CV (%)	22.30	20.89	8.06	8.46	15.44	14.18

 Table 4. Effects of media and seed pre-treatment on percentage germination.

Values with the same alphabet (s) along the vertical column are not significantly different (P = 0.05).

 Table 5. Mean values of some growth parameters of seedlings grown for 6 weeks in various media.

Media	Mean leaf number	Mean hypocotyls length (cm)	Mean epicotyls length (cm)	Mean leaf area (cm)
GS	9.4b ^c	11.2 ^c	10.0 ^d	173.2 ^d
SD	9.0 ^c	10.0 ^d	9.4 ^d	176.2 ^{cb}
GS + SD	9.5 ^b	11.4 ^{bc}	10.6 ^{cd}	176.5 [°]
GS + PD	10.4 ^b	12.66	11.0 ^{bc}	180.4 ^b
SD + PD	10.2 ^b	12.0 ^b	10.5 [°]	178.6 ^{bc}
GS + PD + SD	12.6 ^a	14.3 ^a	12.0 ^a	184.5 ^a
x	10.18	11.92	10.5 ⁸	178.23
SE	1.18	1.33	0.81	3.57
CV (%)	11.60	11.16	7.66	200

Values with the same alphabets along the vertical column are not significantly different (P = 0.05).

Observations on the effects of seed pre-treatment on seed germination showed that the earliest seed germination occurred 2 weeks after sowing with SB₃, SB₂, and SB₅ seed batches, sown in the mixed media (GS + PD + SD, GS + SD, SD + PD and GS + PD). The same seed batches took about 3 weeks to germinate in SD medium and 4 weeks in GS medium. Irrespective of media, SB_4 and SB_6 seed batches failed to germinate seed germination. Seed germination lasted for 1 to 3 weeks. As summarized in Table 4, the effects of seed pre-treatment and media on percentage seed Generally, higher germination showed variations. percentage seed germination was obtained with seeds sown in the mixed than in the single media. Within the media, the highest percentage seed germination was obtained when seeds were sown in GS + PD + SD medium, while the least was obtained with seeds sown in GS medium.

Within the pre-treated seeds, SB_3 (Seeds that were washed in tap water, soaked in hot water (50°C) for 15 min before sowing) consistently had the highest percentage germination responses in all the media, while the control (Un-pretreated seed), had the least.

Comparatively, the highest percentage seed germination was obtained when seeds were soaked for 15 min. In hot water (50°C), air dried and sown in 1:2:1 mixture of GS + PD + SD medium.

Data obtained from growth and development of some seedling aerial parameters (Table 5) showed that seedlings grown in the mixed media had higher values of the analyzed growth parameters than those grown for the same period in the single media. The results further showed that seedlings grown in 1:2:1 mixture of GS + PD + SD medium consistently had the highest values of all the analyzed aerial growth parameters, while seedlings grown for the same period in SD medium had the least.

DISCUSSION

Physicochemical properties of the 6 media showed variations, with the mixed media having higher water holding capacity and nitrogen content than the single media. The results agreed with earlier numerous reports on the qualities of good potting media, Smith (1998) reported that single media have numerous macropores

that encourage water loss through drainage, hence do not retain much of the absorbed water. The author further recommended that single media like SD and GS can be made to retain more water by amending them with organic matter which will reduce the macropores to a lot of micropores, noted for aeration and encouraging retention of absorbed water. Similar reports, the amendment of potting medium for high water holding capacity have been reported (Macmillan 1991; Wilson et al., 2001; Baiyeri and Mbah, 2006).Thus; in this study the presence of micropores in the mixed media appear to account for their superior water holding capacity over that of the single media.

Detailed information on the species seed germination appears scanty. The species has not been successfully cultivated but protected. Ofori (1982) reported that the seeds normally germinate 2 to 4 weeks after sowing but gave no details. In this study, pre-treated seeds derminated within 1 week and the un-pretreated seeds 2 to 3 weeks after sowing. The germination period of the un-pretreated seeds agreed with earlier reports (Ofori, 1982) while that of the pretreated seeds showed reduction. The drastic reduced germination period of the pre-treated seeds observed in this study appear influenced by the various pretreatments. Naturally the species appear to have ecological adaptive strategies that allow it to reproduce and establish in nature but pose problems in its domestication: Firstly, the tree attains great height (over 50 m) before fruiting (Keay et al., 1964) thus, making it difficult to harvest its fruits. Secondly, the seeds are surrounded by sticky, oily substances which could impose seed dormancy. Many authors reported that the presence of oily substances surrounding seeds delay seed germination (Mbakwe and Nzekwe, 2005; Nzekwe and Uju, 2008). Naturally, Bats, Chyroptera spp. feed on the fruits and later release the seeds together with their fecal matters. The seeds later germinate and then establish as favourable conditions prevail. It appears that the seeds are cleared of the dormancy imposing factor as they (seeds) pass through the digestive tract of the bats. Thus, the reduced period of germination of the pre-teated seeds (SB₂ SB₃ and SB₅) appear explained, possibly because the oily substances could be soluble in both cold and hot water as applied. The long period of germination of the un-treated seeds could be attributed to the period taken by the media to be saturated with water to an extent that the dormancy factors could be solubilized and removed. The results also showed that seeds soaked in hot water was left to rest till water cooled before sowing SB₄, SB₆ (50° and 60°, respectively) did not germinate irrespective of media. This implied that the pre-treatments were hash and unfavourable for M. excelsa seed germination.

The effects of pre-treatment and media showed that higher percentages of seed germination were obtained when the variously pretreated seed were sown in the mixed than in the single media. The results agreed with those of earlier authors on pre-treatment of seeds (Onvekwelu, 1990) and on pre-treatment of seed and media (Mbakwe and Nzekwe, 2005; Baiyeri and Mbah, 2006; Nzekwe and Uju, 2008). In this study, seed pretreatment removed seed dormancy factor, while mixed media, by the virtue of their high water holding capacity created water vapour saturated atmosphere around the seeds, thus ensuring for regular water imbibitions by the seeds. Similar reports on media water vapour saturated atmosphere on seed germination have been made (Nwankwo, 1984; Nzekwe et al., 2002; Nzekwe, 2002; Baiyeri and Mbah, 2006). Low percentage seed germination in the single media (GS and SD) is explained by their low water holding capacity. It appeared that some seeds sown in them lost viability due to the long period the media stayed before being saturated with water. Smith (1998) pointed out that, single media take long period to provide favourable environment for seed dermination due to their numerous macropores and low water holding capacity.

Within media and irrespective of pre-treatments, 1:2:1 mixture of GS + PD + SD medium exerted great influence in the production of the highest percentage seed germination responses. The results also showed that SB₃ (seeds soaked for 15 min in hot water 50°Cand sown in 1:2:1 mixture of GS + PD + SD medium had the highest percentage seed germination. There are no earlier reports on pre-treating the seeds of M. excelsa and sowing in different media, thus making the results of this study useful in the propagation of the species by the seeds. The high percentage seed germination (92% ± 11.17) obtained implied that the technique be adopted for routine propagation of the species. The technique is simple, materials used are cheap, readily available, hence the technique can be readily adopted by nursery men and foresters, whose contributions in the conservation of *M. excelsa*, currently threatened, cannot be ignored.

The large quality of seedlings obtained by this technique underscores the reports that the species propagated vegetatively by grafting air layering rooting root cutting (Ofori, 1982) and by rooting leafy stem cuttings (Ofori et al., 1996). Vegetatively propagated plant species have benefits, but are associated with early branching and above all, drastic reduced growth in height (Okafor, 1983; Hartuiank and Kerster, 1983; Mbakwe, 2005). Vegetative propagation is without doubt good for ornamentals and edible fruit trees but unsuitable for timber yielding species.

Nwoboshi (1976) observed that 60 out of 560 timber yielding species identified by Redhead (1971) can yield timber of commercial value because of their growth height. Hence, propagating *M. excelsa* vegetatively (Ofori, 1982; Ofori et al., 1996) could result in significant loss of substantial volume of wood, the primary desire for growing *M. excelsa*. Keay (1989) pointed out that, the species grows up to 50 m unbranched thus accounting for its large wood volume.

Mean values of some aerial growth parameters of M. excelsa seedlings grown for 6 months (Seedling height, collar giveth, hypocotyl and epicotyl height, respectively, leaf number and leaf area) showed trend. Seedlings grown in the mixed media which had higher percentage nitrogen content also had higher values of the growth parameters than the seedlings grown for the same period in the single media. The results suggest that the single media were impoverished, not only due to their low nitrogen contents but also for other plant chemical nutrients (not analyzed). Several authors have given detailed role of nitrogen in plant growth and development. The authors pointed out that nitrogen favours the growth and development of the aerial parts of plants more than the root system (Bidwell, 1979; Hartmann and Kester, 1983; Smith, 1998). In this study, 1:2:1 mixture of GS + PD + SD medium which has the highest percentage nitrogen content also influenced the growth and development of seedlings that had the highest mean values of all the parameters analyzed. The results thus implied the production of uniform, vigorously growing seedlings, needed for the conservation of the species. The results further suggest that inclusion of poultry droppings in the formulation of potting media can be relied upon for the production of vigorously growing seedlings.

Based on the findings of this study, it can be conclude that: *M. excelsa* can be propagated by the seeds. That the medium 1:2:1 mixture of GS + PD + GS, which has high water holding capacity and high nitrogen content can be relied upon for obtaining very high percentage seed germination, when the species seeds were soaked for 15 min in hot water (50°C) before sowing.

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