RESPONSES OF *Monodora myristica* (Gaertn.) Dunal SEEDLINGS TO SOIL AMENDMENTS AND WATERING REGIMES

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ABSTRACT
This study was carried out in the nursery unit of the department of Forestry and Wildlife Management, Faculty of Agriculture demonstration farm, University of Port Harcourt, to examine the seedling responses of *Monodora myristica* to soil amendments and watering regimes. The experiment was set up in a completely randomized design with two factors (soil amendments and watering regimes). One hundred and fifty (150) seedlings were transplanted into polybags filled with the different soil amendments. Five soil amendments (Topsoil, topsoil and sawdust, topsoil and ash, topsoil and poultry dung and topsoil and sharp sand) and three watering regimes (once in a week, twice in a week, and thrice in a week) were used. A two-way analysis of variance was used to assess the effects of the factors (watering regimes and soil amendment) and their interaction on growth parameters and Duncan multiple range test at $P \leq 0.05$ was used for mean separation. Result from this study indicated that there were significant differences($p \leq 0.05$) on the effects of sowing media and watering frequency on growth parameters (seedling height, collar diameter and leaf number) while their interaction (sowing media x watering frequency) was not significantly different ($p > 0.05$). The result of the effect of sowing media and watering frequency on seedling height indicated that seedlings grown in topsoil displayed better growth in height, mixture of topsoil and saw dust displayed better growth in collar diameter; while mixture of topsoil and sharp sand displayed better growth in leaf number. Worst growth parameters were observed in seedlings grown in a mixture of topsoil and ash. In addition, seedling height, collar diameter and leaf number decreased continuously with decrease in watering frequency. Also, the result of the effect of sowing media and watering frequency on biomass accumulation was also significant but their interaction was not significant. The findings imply that *M. myristica* seedlings grow better when watered thrice a week. Also, Topsoil enhanced the growth of Monodora with regards to better biomass accumulation and height. Therefore, the thrice a week watering regime and the use of topsoil are recommended for improved growth of *M. myristica* seedlings.

KEYWORDS: *Monodora myristica*, Seedlings, Soil Amendments, Watering Regimes

INTRODUCTION
Monodora myristica (Gaertn.) Dunal commonly known as "Eghuru'' in Igbo is a perennial edible plant of the evergreen and deciduous forest that belongs to the family of Annonaceae (Burabai *et al.,* 2007). It is found mostly in the evergreen forests of West Africa in countries like Nigeria, Liberia, Cameroon, Uganda, West Kenya and Angola (Weiss, 2002) and its common names are African nutmeg, Calabash nutmeg, Eghuru, Ariwo, Ehiri and Airama (Uwakwe and Nwaoguikpe, 2008). It grows up to 35m high and 2m in girth and have flowers that are conspicuous, attractive and scented (Uyoh *et al.*,2014). The bole is usually clear, the wood is white or greyish, hard, somewhat tough and does not split well; the wood is suitable for carpentry, house fittings, joinery and walking stick while the seeds are also made into necklaces (Erukainure *et al.,* 2012). Its subspherical fruit may attain a diameter of 20cm; also, the seeds are embedded in a white sweet-smelling pulp and are the most economically important part of the tree (Agiriga and Siwela, 2017).

Monodora myristica is a very useful but neglected tropical tree that is distributed in many countries of the tropics and subtropics (Dike, 2010). The different parts of this plant such as the flowers, bark and seeds contain important minerals and are good sources of Vitamins, Amino acids, proteins and various phenolics; they also contain bioactive substances that exhibit health-beneficial effects, including stimulation of the cardiac and circulatory system, antibacterial activities, anti-inflammatory, antispasmodic, diuretic, antihypertensive, antioxidants, anti-diabetic, cholesterol lowering, hepatoprotective and antifungal activities (Agiriga and Siwela, 2017). The bark is used to treat stomach-ache, haemorrhoids and febrile pains whereas the seeds are used in Africa as a substitute for nutmeg, hence the name Calabash nutmeg (Koudou *et al.,* 2007).They are used as an aromatic and stimulating addition to medicines and to snuff; the seeds contain 5-9% of a colourless essential oil consisting largely of terpenes and with a pleasant taste and smell and also have about 35-36% of a reddish-brown fixed oil which is mainly linoleic acid and 35% oleic acid (Celtnet

Recipes, 2011). They are endowed with magical attributes for which they are valued in many medical preparations (Ajayi *et al.*, 2004).

The performance of seedlings in the field is determined by their performance in the nursery (Adelaja and Fasidi, 2008). Good production of permanent tree crop seedlings in the nursery phase is highly influenced by the nursery soil used (Obiefuna and Ibeawuchi, 2012). According to Agbo and Omaliko (2006), nursery soil mixtures have been found to influence the quality of seedlings produced. Use of suitable growing media or substrate is essential for production of quality plant species (Agbo and Omaliko, 2006). It directly affects the development and later maintenance of the extensive functional rooting system (Baiyeri, 2006). A good growing media would provide sufficient anchorage or support to the plant, serves as reservoir for nutrient and water, allow oxygen diffusion to the roots and permit gaseous exchange between the roots and atmosphere outside the root substrate (Abad *et al.,* 2002). The quality of seedlings obtained from a nursery influences re-establishment in the field and its eventual productivity (Baiyeri, 2006).

Based on the foregoing, it becomes necessary to find out the most appropriate nursery medium to enhance seedling growth in the nursery, subsequent field establishment, growth and yield of *M. myristica.*

In a similar vein, water availability is the most important environmental factor known to have strong influence on tree species and distribution in the tropics (Bongers *et al.*, 2004). Soil moisture is the water that is held in the spaces between soil particles (Aderounmu *et al.,* 2017). Moisture especially, brings seeds back to life (Keller, 2005). When the seeds absorb water in a process called imbibition, it activates enzymes to initiate the germination process (Akubuili, 2013). Water is an important natural resource that supports life and growth of plants, but there is a growing concern on water availability especially in dry land forestry and nursery raised seedlings (Goyne and Mclntyre, 2003). The availability of permanent water supply has been one of the major challenges in plant nursery establishment and management, especially in the drier regions (Daba and Tadese, 2017). Water is of great importance to the growth of plants because; it controls the rate of transpiration which in turn has effect on the inflow of nutrient solutions (Aderounmu *et al.,* 2017). Growth and biomass production is directly proportional to the supply and use of water in plant (Olajuyigbe *et al.,* 2012). It is also an important factor in the growth, development and productivity of plants (Gbadamosi, 2014; Ogidan *et al.*, 2018). Isah *et al.* (2012) and Gbadamosi (2014) stated that water is required by plants for the manufacture of carbohydrates and as a means for transportation of foods and mineral elements. Hence water is vital to the success of seedling production especially when large quantities are required for afforestation and reforestation programmes (Oboh and Igharo, 2017). Daba and Tadese (2017) also noted that sufficient quantity and quality of water is extremely important for the production of tree seedlings at nursery site. For tree nurseries, regular watering like 2 – 3 times a week is necessary to produce good quality seedlings. This is because any stagnation in seedling growth or subsequent mortality, translates into economic loss to a nursery operator (Mhango *et al.*, 2008; Mng' omba*et al.*, 2011; Oboh and Igharo, 2017). On the order hand, too much water can cause seeds to rot instead of developing into a seedling (Bratasevec, 2013).

The biodiversity of Nigeria's rainforests is disappearing at a fast rate due to the increased pressure on the ecosystem, as a result of the increase in human populations (Walker, 1994). Other factors like high rate of urbanization, deforestation, increasing mobility, and development of new housing schemes which result in the large-scale destruction of the natural forests, are threatening the continued existence of many species (Adelaja and Fasidi, 2008). *M. myristica* is a useful but neglected tropical tree that is distributed in many countries of the tropics and subtropics (Ekeanyanwu *et a*l., 2009). However. Its populations are disappearing at an alarming rate in Nigeria due to pressure from rising human populations (Walker, 1994).

Another problem *M. myristica* is facing is that it is largely harvested from the wild and greatly affected by forest fire, reckless and uncontrolled felling of trees for timber and fuel wood without replanting ((Mabberley, 2008). Hence, the need for its preservation in order to prevent it from going into extinction (Adelaja and Fasidi, 2008). Furthermore, there is inadequate information on the effect of soil amendment and watering regimes on the growth of *M. myristica* seedlings for regeneration purposes, thereby creating the need for this study. This study therefore investigated the seedling responses of *Monodora myristica* to soil amendment and water stress with a view to providing knowledge that would support the artificial regeneration and cultivation of *M. myristica*.

MATERIALS AND METHODS

Experimental site

The study was carried out at the forest nursery of the Department of Forestry and Wildlife Management., Faculty of Agriculture, University of Port Harcourt, Rivers State, between November 2021 and May 2022. The nursery lies at Latitude 04°53˃38.3'N and Longitude 00.6° 54' 38" E.

Materials

The following materials were used for the study;

1. Viable seeds of *Monodora myristica* collected from a healthy mother tree.

2. Germination trays used for germination and polypots for growth.
3. Meter rule for measurement of seedling height.
4. Digital vernier calliper for measuring collar diameter of seedlings.
5. A field notebook and pen for data collection.
6. Oven for drying of seedlings
7. Weighing scale for seedling weight measurement

Fruit collection and processing

Mature fruits of *Monodora myristica* were harvested from a healthy mother tree. The fruits were depulped manually so as to avoid damaging the seed. The floatation method of seed viability test was employed to detect the viable seeds. Seeds that floated after putting them in a container filled with water were regarded as non-viable and discarded while the seeds that did not float were regarded as viable and used for the experiment.

Experimental Design

The experiment was set up in a completely randomized design (CRD) with two factors (soil amendments and watering regimes). Three hundred (300) randomly selected seeds were directly sown in a germination tray filled with sharp sand. Watering was done daily and seeds were germinated under shade. No fertilizer or bacterial and/or mycorrhizal inoculation was used. After one month of establishment, one hundred and fifty seedlings were transplanted from germination trays into polybags filled with different soil amendments. Five sowing media (Topsoil, topsoil and sawdust, topsoil and ash, topsoil and poultry dung and topsoil and sharp sand) and three watering regimes (once in a week, twice in a week, and thrice in a week) (i.e. 10 seedlings x 5 soil amendments x 3 watering regimes = 150 experimental units) were used. Soil amendment materials were mixed with topsoil at a ratio of 3:1. Prior to the transplanting operation, the sowing media were fully watered to field capacity to maintain the moisture content of the soil. Weeding was carried out regularly and when required throughout the period of the experiment.

Data collection

Seedling growth

Shoot parameters measurement was done on all seedlings from month one after treatment application and monthly thereafter for six months. Seedling height was measured from the substrate level to the tip of the youngest leaf using a meter rule; stem collar diameter was measured at the root collar using a digital calliper; while leaf and branch production were determined by directly counting the number of leaves and branches.

Biomass Determination

At the end of the experiment, five seedlings per treatment were carefully removed from the pots and the root system exposed by carefully washing off excess growth media from the roots, absorbent paper was used for blotting excess moisture from the plants. Seedlings were separated into shoot and root components; separation was at the soil line. The fresh weight of shoot (including the leaves) and root were taken and then placed in a paper bag for drying. The samples were oven dried at 70°C for three days (72 hrs.).

Seedling shoot and root (fresh and dry) weights were measured using digital weighing scale. Shoot and root dry weights were used to determine; total dry weight, moisture content and root to shoot (RSR) ratio using the following equation.

Total Dry Weight (TDW) = Shoot dry weight + root dry weight

Moisture Content (MC) = Fresh weight – Dry weight

Root to Shoot Ratio (RSR) $= \frac{\text{Total root Dry Weight}}{\text{Total Shoot Dry Weight}}$

Data Analysis

A two-way analysis of variance was carried out on the growth data to assess the effects of the fixed factors (watering regimes and soil amendment) and their interaction on growth parameters. Duncan multiple range tests were used to indicate levels of differences and compare means of the same watering regimes under various soil amendments. Data that were collected on seedling growth was analysed using SPSS statistical software (SPSS version 21, SPSS Inc.).

RESULTS

Effect of sowing media and watering frequency on the growth of *Monodora myristica*

A two-way anova indicated significant effects ($p \leq 0.05$) of sowing media and watering frequency on seedling height, collar diameter and leaf production of *Monodora myristica*. The interaction between these effects was not significant ($p > 0.05$) (Table 1).

Table 1. Two-way ANOVA of the effects of sowing media and watering frequency on the growth of *Monodora myristica*

		Seedling Height (cm)		Collar Diameter (mm)		Leaf Number	
	d.f.	*F cal*	*P value*	*F cal*	*P value*	*F cal*	*P value*
Sowing Media	4	6.491	.000	4.113	.004	7.087	.000
Watering Frequency	2	10.219	.000	7.570	.001	5.110	.007
Sowing Media x Watering Frequency	8	.136	.997	.192	.992	1.363	.218

Seedling height

Seedlings grown in topsoil had better growth in height (21.463 cm) when compared with seedlings grown in other media while seedlings grown in a mixture of topsoil and ash displayed worst growth in height (17.913 cm) at the different watering frequencies. Also, seedling height decreased continuously with decrease in watering frequency (Table 2).

Table 2. Effect of sowing media and watering frequency on seedling height at 6 months

Watering	Sowing Media					
Frequency	TS	TS/SD	TS/ASH	TS/SS	TS/PD	Mean
Once Weekly	20.56±0.94	19.16±0.94	16.55±0.94	18.70±0.94	17.78±0.94	18.55±0.42b
Twice Weekly	21.14±0.94	20.77±0.94	18.21±0.94	21.05±0.94	19.23±0.94	20.08±0.42a
Thrice Weekly	22.69±0.94	22.01±0.94	18.98±0.94	21.74±0.94	20.77±0.94	21.23±0.42a
Mean	21.46±0.55a	20.65±0.55ab	17.91±0.55c	20.50±0.55ab	19.26±0.55bc	

Means with the same alphabet do not differ significantly from each other at p > 0.05

TS = Topsoil; SD = Saw Dust; SS = Sharp Sand; PD = Poultry Dung

Collar diameter

Highest collar diameter was observed in seedlings grown in a mixture of topsoil and sawdust, followed by seedlings in topsoil while seedlings grown in a mixture of topsoil and ash displayed worst growth in collar diameter. Collar diameter also decreased continuously with decrease in watering frequency (Table 3).

Table 3. Effect of sowing media and watering frequency on seedling collar diameter of at 6 months

Watering	Sowing Media					
Frequency	TS	TS/SD	TS/ASH	TS/SS	TS/PD	Mean
Once Weekly	2.690±0.14	2.760±0.14	2.250±0.14	2.680±0.14	2.610±0.14	2.598±0.06b
Twice Weekly	2.810±0.14	2.820±0.14	2.500±0.14	2.710±0.14	2.870±0.14	2.742±0.06b
Thrice Weekly	3.020±0.14	3.140±0.14	2.680±0.14	2.950±0.14	2.940±0.14	2.946±0.06a
Mean	2.840±0.08a	2.907±0.08a	2.477±0.08b	2.780±0.08a	2.807±0.08a	

Means with the same alphabet do not differ significantly from each other at p > 0.05

TS = Topsoil; SD = Saw Dust; SS = Sharp Sand; PD = Poultry Dung

Leaf Production

Highest leaf production was observed in seedlings grown in a mixture of topsoil and sharp sand, followed by seedlings grown in a mixture of topsoil and sawdust while seedlings grown in a mixture of topsoil and ash displayed worst growth in leaf production. Leaf production also decreased continuously with decrease in watering frequency (Table 4).

Table 4. Effect of sowing media and watering frequency on leaf production at 6 months after sowing

Watering	Sowing Media					
Frequency	TS	TS/SD	TS/ASH	TS/SS	TS/PD	Mean
Once Weekly	3.500±0.27	3.000±0.27	2.000±0.27	3.400±0.27	2.600±0.27	2.900±0.12b
Twice Weekly	2.900±0.27	3.600±0.27	2.500±0.27	3.700±0.27	3.300±0.27	3.200±0.12ab
Thrice Weekly	3.500±0.27	3.400±0.27	3.000±0.27	3.700±0.27	3.600±0.27	3.440±0.12a
Mean	3.300±0.16a	3.333±0.16a	2.500±0.16b	3.600±0.16a	3.167±0.16a	

Means with the same alphabet do not differ significantly from each other at p > 0.05

TS = Topsoil; SD = Saw Dust; SS = Sharp Sand; PD = Poultry Dumg

Effect of sowing media and watering frequency on biomass accumulation of *Monodora myristica* seedlings

A two-way anova indicated significant effects ($p \leq 0.05$) of sowing media and watering frequency on biomass accumulation of *Monodora myristica*, but the interaction of these effects on shoots dry weight, root dry weight and total dry weight was not significant ($p > 0.05$) (Table 5).

Table 5: Two-way anova of the effects of sowing media and watering frequency on biomass accumulation of *Monodora myristica* seedlings

		SDW (g)		RDW (g)		TDW(g)	
	d.f.	*F cal*	*P value*	*F cal*	*P value*	*F cal*	*P value*
Sowing Media	4	23.707	.000	5.892	.000	17.676	.000
Watering Frequency	2	11.640	.000	6.569	.003	10.881	.000
Sowing Media x Watering Frequency	8	.960	.476	.828	.582	.832	.578

Shoot dry weight

Highest shoot dry weight was observed in seedlings grown in topsoil, followed by seedlings grown in a mixture of topsoil and sawdust while seedlings grown in a mixture of topsoil and ash had the lowest shoot dry weight. The shoot dry weight decreased continuously with decrease in watering frequency (Table 6).

Root dry weight

Seedlings grown in topsoil had highest root dry weight, followed by seedlings grown in a mixture of topsoil and sawdust while seedlings grown in a mixture of topsoil and poultry dung had the lowest root dry weight. Root dry weight also decreased continuously with decrease in watering frequency (Table 6).

Total dry weight

Highest total dry weight was observed in seedlings grown in topsoil, followed by seedlings grown in a mixture of topsoil and sawdust while seedlings grown in a mixture of topsoil and ash had the lowest total dry weight. The total dry weight also decreased continuously with decrease in watering frequency (Table 6).

Table 6. Effect of sowing media and watering frequency on plant biomass of *Monodora myristica* seedlings

Watering Frequency	Sowing Media					
	TS	TS/SD	TS/ASH	TS/SS	TS/PD	Mean
Shoot Dry Weight (g)						
Once Weekly	0.354±0.03	0.294±0.03	0.118±0.03	0.322±0.03	0.220±0.03	0.262±0.02c
Twice Weekly	0.376±0.03	0.410±0.03	0.200±0.03	0.364±0.03	0.214±0.03	0.313±0.02b
Thrice Weekly	0.448±0.03	0.454±0.03	0.226±0.03	0.358±0.03	0.322±0.03	0.362±0.02a
Mean	0.393±0.02a	0.386±0.02a	0.181±0.02c	0.348±0.02a	0.252±0.02b	
Root Dry Weight (g)						
Once Weekly	0.164±0.02	.152±0.02	0.124±0.02	0.136±0.02	0.132±0.02	0.142±0.01b
Twice Weekly	0.184±0.02	.174±0.02	0.150±0.02	0.158±0.02	0.100±0.02	0.153±0.01b
Thrice Weekly	0.200±0.02	.216±0.02	0.146±0.02	0.180±0.02	0.156±0.02	0.180±0.01a
Mean	0.183±0.01a	.181±0.01a	0.140±0.01b	0.158±0.01ab	0.129±0.01b	
Total Dry Weight (g)						
Once Weekly	0.518±0.05	0.446±0.05	0.242±0.05	0.458±0.05	0.352±0.05	0.403±0.02c
Twice Weekly	0.560±0.05	0.584±0.05	0.350±0.05	0.522±0.05	0.314±0.05	0.466±0.02b
Thrice Weekly	0.648±0.05	0.670±0.05	0.372±0.05	0.538±0.05	0.478±0.05	0.541±0.02a
Mean	0.575±0.03a	0.567±0.03a	0.321±0.03b	0.506±0.03a	0.381±0.03b	

Means with the same alphabet do not differ significantly from each other at $p > 0.05$

TS = Topsoil; SD = Saw Dust; SS = Sharp Sand; PD = Poultry Dung

DISCUSSION

The result of this study indicated significant effects ($p \leq 0.05$) of watering frequency on seedling height, collar diameter and leaf production of *Monodora myristica*. The findings are supported by Fredrick *et al.* (2018) and Isah *et al.* (2012) who observed highly significant differences in *Anonna muricata* seedlings and *A. senegal* provenances respectively under different watering regimes. This result also concurs with that of Mohamed *et al.* (2013) who observed significant differences in height and leaf number among all irrigation frequencies in five tropical species. Olajide *et al.* (2014) also reported a significant difference in *Dialium guineense* seedlings stem collar diameters subjected to different watering regimes while Gbadamosi (2014) reported significant difference in seedling height of *Picralima nitida* subjected to different watering regimes. However, the result disagrees with the findings of Sale (2015) who reported non-significant differences in seedling height and leaf number of *Parkia biglobosa*. It also disagrees with the report of Mohamed *et al.* (2013) and Gbadamosi (2014) who observed non-significant differences in collar diameter of five tropical species and *Picralima nitida*, respectively, subjected to different watering regimes. Water stress causes significant variation in seedlings relative growth rate (Abdelbasit *et al.*, 2012). Significant variation in both morphological and physiological adaptation to water stress in tree species has been reported by (Cregg, 1994). The highest seedling indices (height growth and leaf number) found in seedlings watered three times suggest that adequate water is necessary for *Monodora myristica* seedlings to grow and develop properly since it had a more positive impact on seedlings when compared to other treatments. Various vital processes in plants including cell division, cell elongation, stem as well as leaf enlargement and chlorophyll formation depend on plant water availability (Price *et al.*, 1986). This probably explains why most frequently watered seedlings gave best growth in seedling height, collar diameter and leaf number.

The significant effects ($p \leq 0.05$) of sowing media on seedling height, collar diameter and leaf production of *Monodora myristica* agree with previous findings by Keyagha *et al.* (2016) and Fredrick *et al.* (2018) who observed significant difference in the growth and development of *Irvingia wombolu* and *Annona muricata* seedlings respectively, subjected to different media and Baiyeri (2003) who reported significant effects of nursery media on seedling emergence and early seedling growth of two tropical tree species. Highest seedling height observed in topsoil could be because topsoil contains more of both micro and macro nutrients required by plants for their normal growth and developmental activities. Also, better growth in collar diameter observed in mixture of topsoil and sawdust and leaf production observed in mixture of topsoil and sharp sand may be due to improved physical status of these combinations (Osaigbovo *et al.*, 2010)

Better plant biomass observed in topsoil could be related to high water retention capacity of the media (Fredrick *et al.*, 2017) and is an indication that it is very suitable for the growth of *Monodora myristica* with regards to biomass accumulation. Poor growth observed in mixture of topsoil and ash agrees with the report of Keyagha *et al.* (2016) who also noted that topsoil and wood ash mixture exhibited lowest seedling growth parameters when compared with other treatments used in their study.

The non-significant effects ($p > 0.05$) of the interaction of sowing media and watering frequency on seedling height, collar diameter and leaf production of *Monodora myristica*as found in this study, disagrees with that of Dawid (2020) who reported a highly significant effect of the interaction of sowing media and watering frequency on seedling height of *Aframomum cororima.* The difference in the results of both studies may be as result of differences in the physiological characteristics and requirements of the species.

CONCLUSION AND RECOMMENDATION

The findings imply that *Monodora myristica* seedlings grow better when watered thrice a week, followed by twice a week and with these findings, there is every possibility that the seedlings will grow better when watered frequently. This means that *Monodora myristica* seedlings cannot be raised under some form of water stress since frequent watering enhanced the growth of the seedlings. Also, topsoil enhanced the growth of Monodora with regards to better biomass accumulation and height. Therefore, the thrice a week watering regime and the use of topsoil are recommended for improved growth of *Monodora myristica* seedlings.

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