



Organic fertilizers and watering frequency effects on *Tetrapleura tetraptera* seedlings survival and growth in nursery



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Article History

Received 13 February, 2023
Received in revised form 09
March, 2023
Accepted 14 March, 2023

Keywords:

Growth,
Organic fertilizers,
Seedlings,
Tetrapleura tetraptera,
Water deficiency.

Article Type:

Full Length Research Article

ABSTRACT

Tetrapleura tetraptera (Schum and Thonn) is a multipurpose tree species of tropical Africa whose population is declining due to seed dormancy, over-exploitation, and inadequate information on silvicultural techniques for domestication and conservation. This study aimed to determine the effect of organic fertilizers and watering frequencies on seedlings survival and growth in order to improve *T. tetraptera* seedling growth without wasting water resources. A factorial experimental design of two factors and three replications was adopted. The first factor was formed by 3 types of substrates and the second by 5 watering frequencies. Seedlings' height and stem collar diameter were measured, and the number of leaves and permanently withered seedlings were recorded weekly. The data collected were processed with an Excel 2020 spreadsheet and statistical analyses consisting of a two-way analysis of variance followed by a Tukey test were carried out with R version 4.0.3. The study revealed that, regardless of the substrates, the highest mortality rate was recorded for the watering frequency corresponding to once every 5 days. The sandy loam substrate without cow dung contributed to most mortality cases as well as being the substrate with the highest mortality rate. The statistical tests showed significant differences between the effect of substrates and watering frequencies taken separately on seedling height and stem collar diameter growth leading to the conclusion that, *T. tetraptera* seedlings perform well on fertilized substrates with watering frequencies ranging from once every 3 or 2 days. The perspective of inoculation as a way of further improving seedlings' survival and growth has been discussed.

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INTRODUCTION

Natural ecosystems such as forests and savannahs are home to an important number of biological diversities formed by plants and animals that provide a wide range of

services including biodiversity habitat, climate change mitigation, soil erosion control, water, and organic matter cycling (Salem and Mercer, 2012; Estoque et al., 2018). In Tropical Africa, these ecosystems supply to urban and rural population food products and medicines for subsistence and trade in addition to construction materials (Hernández-Morcillo et al., 2013; Wu, 2013).

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Tetrapleura tetraptera (Schum and Thonn) commonly known as “Prekese” in Ghana, “Aridan” or “Aidan” in Nigeria and “Kikangabalimu” in Uganda, is a deciduous tree species of tropical Africa distributed from Mauritania to Tanzania (Blay, 1997; Omokhua et al., 2015; Kemigisha et al., 2018) whose preferred habitats are savannah, woodlands, dry forest, and riverine forest. However, it is also commonly found in dense rainforests and natural preserved patches of forest around villages. *T. tetraptera* is a flowering plant species native to West Africa and belonging to the family of Fabaceae with a slender bole and low sharp buttresses. It can reach 20 to 25 m in height with a girth of 1.5 to 3 m. Its bark is very thin with a grey-brown colour and a fairly smooth texture. Its leaves are sessile, glabrous or minutely hairy with a common stalk of 15 to 30 cm long. The leaflets on each side of the pinna stalk are slightly elongated, elliptic, or slightly obovate, rounded at both ends. The flowers are pinkish cream turning to orange and crowded in spike like racemes of 5 to 20 cm long, usually in pairs in the upper leaf axils (Ayeni et al., 2021). The fruit, which is very persistent, hangs at the ends of branches, is shiny, glabrous, dark purple-brown, usually slightly curved, with 4 longitudinal, winglike ridges nearly 3 cm broad. Two of these wings are woody and the 2 others filled with soft, sugary pulp, oily and aromatic. The seeds, which rattle in the pods, are small, black, hard, flat, about 8 mm long, embedded in the body of the pod, which does not split open.

Several studies on *T. tetraptera* have revealed the nutritional and medicinal values of its various organs. The fruit and the seeds are used as spice-like ingredients to prepare soup especially for nursing mothers early after childbirth to prevent post-partum contractions (Enwere, 1998). The good flavour and aroma added to food by the fruits and seeds acts like appetizer and increases food consumption desire (Aladesanmi, 2007).

In addition to its use as food products, the aqueous fruit extract has shown hypoglycemic properties (Ojewole and Adewunmi, 2004) and are used for the management of leprosy, convulsions inflammation and rheumatism (Ojewole and Adesina, 1983). The leaves possess strong molluscicidal activity (Omokhua et al., 2015) and are important for the treatment of epilepsy (Akah and Nwaibie, 1993). Its bark is active against cough, bronchitis and is used as a decoction in drinks. When put into vapour bath, the bark is used against rheumatism and fever. The root is utilized for the treatment of gastrointestinal clinical problems (Aladesanmi, 2007). The wood of the species is equally valued in timber and has a fairly hard heartwood (Orwa et al., 2009).

Despite the economic usefulness of *T. tetraptera*, the population of the plant is declining at a disturbing rate due to factors like seed dormancy (Onyekwelu, 1990), over-exploitation and inadequate information on silvicultural techniques (Omokhua et al., 2015). This latter, quite important in species domestication and conservation,

requires investigation about the various factors controlling germination as well as the survival and growth of the species from nursery stage to plantation. Though studies on the species seeds germination have led to significant results about technics to improve seeds germination rates (Omokhua et al., 2015; Usman et al., 2019) only few studies investigated factors that influence the growth of the species. Among them, a study aimed at assessing seed germination potential and early growth performance of *T. tetraptera* seedlings under different light intensities revealed that seeds germination was not significantly influenced by light intensity while growth parameters were. Light intensities of 50% and 75% were found more suitable for the early growth of the species and were recommended for raising *T. tetraptera* seedlings (Fredrick et al., 2020). In almost the same line, another study assessed the effect of varying light intensities and optimum percentage of sunlight favourable for raising *T. tetraptera* seedlings and led to the conclusion that, light intensity of 100% should be used to enhance growth and development of seedlings in nursery (Ayeni et al., 2021). Apart from the impact of light intensity on *T. tetraptera* seedlings growth, the use of green manure and inorganic fertilizer on the growth performance was investigated revealing significant impact of the application of *Leucaenana leucocephala*'s leaves powder as green manure on growth parameters (Akintola et al., 2021). In plantation a study was conducted to determine the efficacy of organic manure on the growth of *T. tetraptera* and found out that oil palm bunch residues, main waste products from oil palm production can be used as an organic fertilizer to improve *T. tetraptera* growth (Aderounmu and Musa, 2019).

However, organic fertilizers encompass both by-products from plants and animals' production. The integration of plant and animal production systems allows the incorporation of wastes from one system to the other to improving and/or optimizing the productivity of the system. The soil types as well as its structural and chemical characteristics are factors that influence the growth and development of plants without mentioning the amount of water in the soil that serves as raising medium or substrate for the plant.

Water is vital for life and survival of all plants as it is essential for the transportation of nutrients and minerals from the soil to the plants and the transport of synthesized products such as carbohydrate through the plants and to the plant's storage organs.

Plants need water for their development and, at this era of climate change and global warming where water resources are becoming scarce, it is important to make sustainable use of the resource to avoid its waste. Therefore, to improve *T. tetraptera* seedlings' growth in nursery without waste of water resources, this study was carried out and aimed at determining the effect of both organic fertilizers and watering frequencies on the species survival and growth.

MATERIALS AND METHODS

Study area

The study was carried out in the city of Ketou located in the Commune of Ketou, Department of Plateau. The Commune of Ketou is located in the South-East of Benin between the latitudes 7°10' and 7°41'17" North, and the longitudes 2°24'24" and 2°47'40" East. It is bordered to the North by the Commune of Save, to the South by the Commune of Pobe, to the East by the Federal Republic of Nigeria and to the West by the Communes of Ouinhi and Zangnanado (Figure 1). It covers an area of 1,775 km² (INSAE, 2014) which represents 1.55% of the national territory and 54.38% of the Department of Plateau. Its climate is characterized by two rainy seasons of unequal duration (March to July and August to November) interspersed by two dry seasons. The annual rainfall varies between 900 and 1,100 mm, and the average annual temperature is from 25 to 29°C with a relative humidity of 31 to 98%. It has a well-drained ferruginous soil where the natural vegetation is characterized by wooded savannahs dominated by *Daniella oliveri*, *Lophira lanceolata* and *Parkia biglobosa*. There are natural patches of forest including the sacred forest of Kouvizoun in Adakplame. The population of the Commune of Ketou is about 157,352 people dominated by the socio-cultural group of Yoruba (INSAE, 2015). Agriculture, trade, logging, and livestock are their main activities.

Experimental design and data collection

A factorial experimental design of two factors and three replications was adopted to carry out the current study (Figure 2). The factors studied were: types of substrates formed by 3 modalities (BKS corresponding to bokashi, SLNWC corresponding to sandy loam soil without cow dung and SLWCD corresponding to sandy loam soil with cow dung) and watering frequencies that comprises 5 modalities (T0 corresponding to permanently wet substrate; T1, T2, T3, and T4 corresponding to watering every two, three, four, and five days, respectively). The watering was done to ensure that the substrate is wet enough without extra water floating above it. For each combined factors of each replication, three seedlings were observed and were used for data collection making a total of 135 *T. tetraptera* seedlings.

On a weekly basis, variables such as height and stem collar diameter were measured, and the number of leaves and permanently withered seedlings were recorded. The height and the stem collar diameter were measured with a tape and an electronic calliper of 0.1 mm of sensitivity respectively. The number of leaves was directly counted, and permanently withered seedlings were identified and

recorded by examining the tissue underneath the seedlings' stem bark. The experiment lasted 10 weeks and measurements were taken at the beginning of every week.

Data processing and analysis

The data collected were entered and processed with Excel 2020 spreadsheet which was also used for illustrative graphs realization. The data collected were used to estimate various parameters such as seedlings survival rate, height average, mean stem collar diameter, and leaf biomass average.

Plants survival was assessed using the mortality rate (Rx) determined according to the formula in Eq1 and calculated for each replication of each treatment formed by the combination of the studied factors. The average of height (Hm), stem collar diameter (Dm) and the leaf biomass (Lm) were calculated to assess these parameters' trend as function of the studied factors over the duration of the experiment using the equations Eq2, Eq3, and Eq4, respectively. To appraise the effect of studied factors on seedlings growth, the current weekly increment in height (CWI_h), stem collar diameter (CWI_d), and leaf biomass (CWI_l) were computed for each replication of every combined studied factors.

$$Rx = \frac{n_{pw} \times 100}{n}$$

Eq1

$$Hm = \frac{1}{n} \sum_{i=1}^n h_i$$

Eq2

$$Dm = \frac{1}{n} \sum_{i=1}^n d_i$$

Eq3

$$Lm = \frac{1}{n} \sum_{i=1}^n l_i$$

Eq4

$$CWI_h = \frac{h_f - h_i}{10}$$

Eq5

$$CWI_d = \frac{d_f - d_i}{10}$$

Eq6

$$CWI_l = \frac{l_f - l_i}{10}$$

Eq7

Where, Rx = Mortality rate (%); Hm = Height average (cm); Dm = Average of stem collar diameter (mm); Lm = Average of leaf biomass (count); CWI_h = Current weekly increment in height (cm/week); CWI_d = Current weekly increment in stem collar diameter (mm/week); CWI_l = Current weekly increment in leaf biomass (count/week); n_{pw} = Permanent withered seedlings (count); n = Total number of seedlings (count); h_i = Height of the seedlings (cm); d_i = Stem collar diameter of the seedlings (mm); l_i = Leaf biomass of the seedlings (count); h_f = Height of the seedlings at the end of the experiment (cm); d_f = Stem collar diameter of the seedlings at the end of the experiment (mm); l_f = Leaf biomass of the seedlings at the end of the experiment (count); h_i = Height of the seedlings at the beginning of the experiment (cm); d_i = Stem collar diameter of the seedlings at the beginning of the experiment (mm); l_i = Leaf biomass of the seedlings at the beginning of the experiment (count).

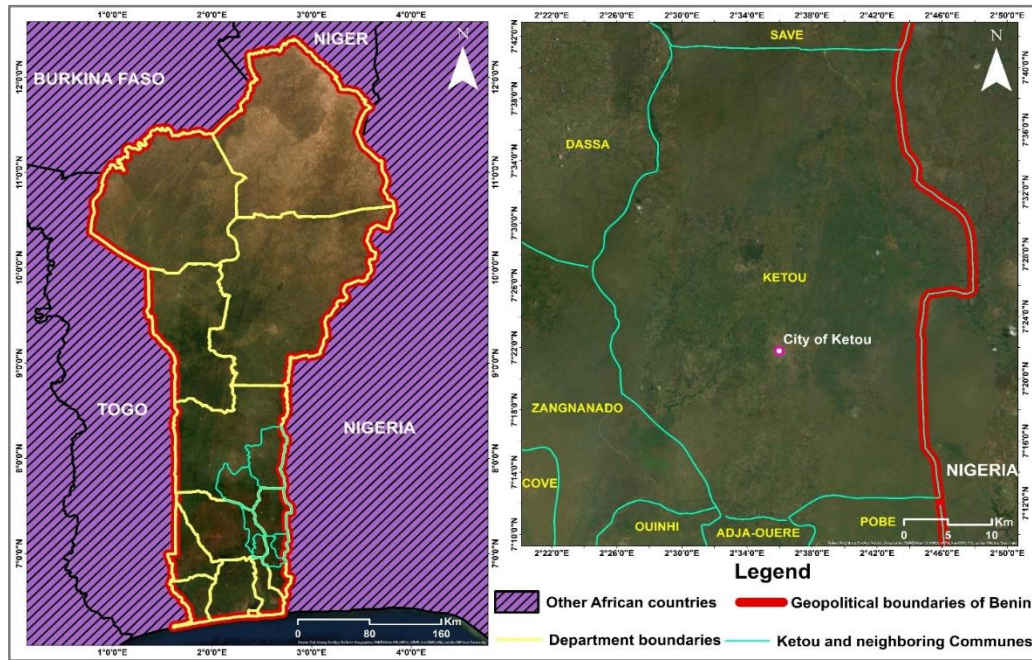


Figure 1. Geographical location of the study area.

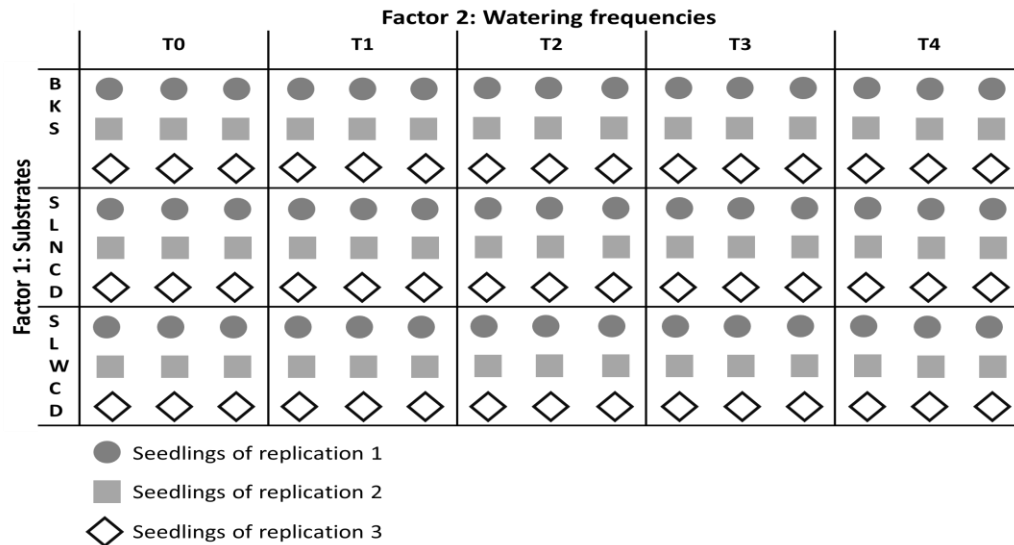


Figure 2. Sketch of the experimental design used to carry out the study. **BKS**, Bokashi; **SLWCD**, Sandy loam with cow dung; **SLNCD**, Sandy loam without cow dung; **T0**, Permanently wet; **T1**, Watering once every 2 days; **T2**, Watering once every 3 days; **T3**, Watering once every 4 days; **T4**, Watering once every 5 days.

Statistical analyses for the identification of significant differences between studied factors were carried out with R software version 4.0.3. A two-way analysis of variance was performed to identify whether there were significant

differences between the type of substrates, the watering frequencies, and their interaction. A Tukey test was used to locate significant differences when the result of the two-way analysis of variance is significant.

RESULTS

Seedlings characteristics at the beginning of the experiment

The seedlings used for the experiment were aged 4 months and the Table 1 presents the average and standard error (SE) of height, stem collar diameter, and number of leaves of the sampled seedlings.

As per the data collected at the beginning and regardless of the studied factors, the height of the seedlings varies from 10.0 to 13.1 cm and the stem collar diameter is ranged from 1.2 to 2.3 mm while the leaf biomass represented by the total number of leaves varies from 7 to 11. Although the variability of the leaf biomass is almost the same for seedlings for all the studied substrates, the lowest variability of the height and stem collar diameter was observed with the seedlings on the sandy loam substrate without cow dung (SLNCD) and the highest for the seedlings on the sandy loam substrate with cow dung (SLWCD) but the bokashi substrate (BKS) has an average variability. When the studied factors are combined, the highest values of the considered variables were globally recorded by seedlings on BKS substrate with one watering every 2 days (13.1 cm in height, 2.1 mm of stem collar diameter, and 11 leaves) and on the substrate SLWCD with one watering every 4 days (12.8 cm in height, 2.3 mm of stem collar diameter, and 9 leaves). It is also on this latter substrate that the lowest values were observed for the watering frequency T4 (10.0 cm in height, 1.2 mm of stem collar diameter, and 10 leaves). Most of the seedlings on SLNCD substrate have the lower values of leaf biomass with the watering frequencies T3, T4 and T2 having the lowest (7 ± 1).

Seedling's survival

Over the ten weeks that the experiment lasted, permanently withered seedlings have been recorded on all the substrates with various mortality rates depending on the watering frequencies applied. The first mortality was recorded for the watering frequency T4 on the substrate SLWCD on the 8th week while for BKS, though the only mortality observed was for the same watering frequency (T4), it was recorded in the last week of the experiment. Contrary to these substrates, mortality cases were observed for all watering frequencies except T0 on the substrate SLNCD. The earliest mortality cases for this substrate were recorded on the 9th week for watering frequencies T4 and T3 and the latest in the last week of the experiment for watering frequencies T2 and T1. Regardless of the substrates, the highest mortality rate (22.2%) was recorded for the watering frequency T4 and the substrate SLNCD contributed for most mortality cases as well as being the substrate with the highest mortality

rate in general (Figure 2). Though, it could be stated that *T. tetraptera* seedlings grown on SLNCD with one watering every 5 days has the highest mortality rate and therefore the lowest survival rate, the two-way analysis of variance conducted on the mortality rates reveals no significant statistical differences between the substrates, the watering frequencies and their interaction as probabilities associated to these tests were greater than 5% (Table 2).

Seedling's growth

Figure 4 shows curves of the evolution of height, stem collar diameter and leaf biomass of seedlings submitted to the various watering frequencies on the studied substrates. While generally, the trends in height and stem collar diameter were continuous and progressive for all the seedlings, it was rather discontinuous for the leaf biomass with successions of gain and loss events. Apart from the height of seedlings submitted to watering frequencies T1, T2, T3, and more or less T0 on the substrate SLWCD that have almost the same growth speed reflected in their roughly parallel curves over the duration of the experiment, most of the watering frequencies especially, T2, T3, and T4 for BKS and T1, T3, and T4 for SLNCD had different growth speed. Regarding the stem collar diameter, it was only on BKS that the seedlings of two watering frequencies T0 and T1 tended to have roughly the same growth speed. It is also important to point out some discontinuities in the stem collar diameter curves trend throughout the weeks of the experiment compared to the height where, it was only with the watering frequencies T1, T3, and T4 on SLNCD that a reduction in seedlings average height due to the death of some dominant seedlings, have been noticed toward the end of the experiment. For the leaf biomass, severe reduction of leaves has been observed the first weeks of the experiment for seedlings on BKS while for the other watering frequencies of the other substrates, the number of leaves has increased.

The assessment studied factors' impacts on the growth performances of *T. tetraptera* seedlings were done by submitting to a two-way analysis of variance the weekly growth between the beginning and end of the experiment for the height, stem collar diameter and the leaf biomass (Table 3). The results showed significant differences between the effects of the substrates and watering frequencies taken separately on seedlings' height growth. Differences were found between all the substrates (Figure 5A) while for the watering frequencies differences existed between T4 - T1, T4 - T2 and T4 - T3 making T4 statistically different from the others watering frequencies. The watering frequencies T1, T2, and T3 were not statistically different from each other and their effect on seedlings' height growth was statistically identical and greater than watering frequency T4 (Figure 5B). The best growth in height (0.18 cm/week) was obtained for SLWCD

Table 1. Sampled seedlings' studies variables characteristics at the beginning of the experimentation.

Factors and modalities		Codes of modalities of factors combined	Height (cm)		Stem collar diameter (mm)		Number of leaves	
Substrates	Watering frequencies		Mean	SE	Mean	SE	Mean	SE
Bokashi (BKS)	T0 (Permanently wet)	BKST0	12.0	0.80	1.7	0.16	10	2
	T1 (Once every 2 days)	BKST1	13.1	0.63	2.1	0.07	11	1
	T2 (Once every 3 days)	BKST2	11.6	1.31	1.6	0.14	9	1
	T3 (Once every 4 days)	BKST3	11.5	1.21	1.4	0.29	10	1
	T4 (Once every 5 days)	BKST4	12.0	0.52	1.5	0.07	9	1
			12.0	0.67	1.6	0.16	10	1
Sandy loam with cow dung (SLWCD)	T0 (Permanently wet)	SLWCDT0	11.8	1.75	1.7	0.37	11	1
	T1 (Once every 2 days)	SLWCDT1	12.6	0.39	1.9	0.19	11	2
	T2 (Once every 3 days)	SLWCDT2	12.0	0.23	1.9	0.15	10	1
	T3 (Once every 4 days)	SLWCDT3	12.8	1.21	2.3	0.09	9	1
	T4 (Once every 5 days)	SLWCDT4	10.0	0.43	1.2	0.04	10	2
			11.8	0.80	1.8	0.21	10	1
Sandy loam with out cow dung (SLNCD)	T0 (Permanently wet)	SLNCDT0	11.7	0.17	1.8	0.16	9	0
	T1 (Once every 2 days)	SLNCDT1	11.4	0.11	2.1	0.13	9	1
	T2 (Once every 3 days)	SLNCDT2	12.0	1.07	1.6	0.13	8	1
	T3 (Once every 4 days)	SLNCDT3	11.5	0.60	1.7	0.24	7	1
	T4 (Once every 5 days)	SLNCDT4	11.1	0.66	2.0	0.06	7	1
			11.5	0.44	1.8	0.14	8	1

SE; Standard error.

Table 2. Results of the analysis of variance of the seedlings' mortality rates.

Studied factors	Degree of freedom	Sum of squares	Mean squares	F value	Pr (>F)
Substrates	2	2419.8	1209.88	3.2667	0.05206
Watering frequencies	4	2716	679.01	1.8333	0.14845
Interaction	8	1284	160.49	0.4333	0.89151
Residuals	30	11111.1	370.37		

Table 3. Results of the two-ways analysis of variance of the studied factors and their interaction with seedlings' height, stem collar diameter, and leaf biomass growth.

Plant variables	Studied factors	Degree of freedom	Sum of squares	Mean squares	F value	Pr (>F)
Height	Substrates	2	0.047904	0.023952	23.6962	6.70E-07
	Watering frequencies	4	0.016223	0.004056	4.0124	0.01006
	Interaction	8	0.015868	0.001984	1.9623	0.08678
	Residuals	30	0.030324	0.001011		
Stem collar diameter	Substrates	2	0.005103	0.002552	12.0256	0.000146
	Watering frequencies	4	0.002602	0.000651	3.0662	0.031315
	Interaction	8	0.007909	0.000989	4.6597	0.000887
	Residuals	30	0.006365	0.000212		
Leaf biomass	Substrates	2	0.4958	0.247898	3.9247	0.03061
	Watering frequencies	4	0.14112	0.03528	0.5586	0.69442
	Interaction	8	0.24447	0.030559	0.4838	0.85789
	Residuals	30	1.89489	0.063163		

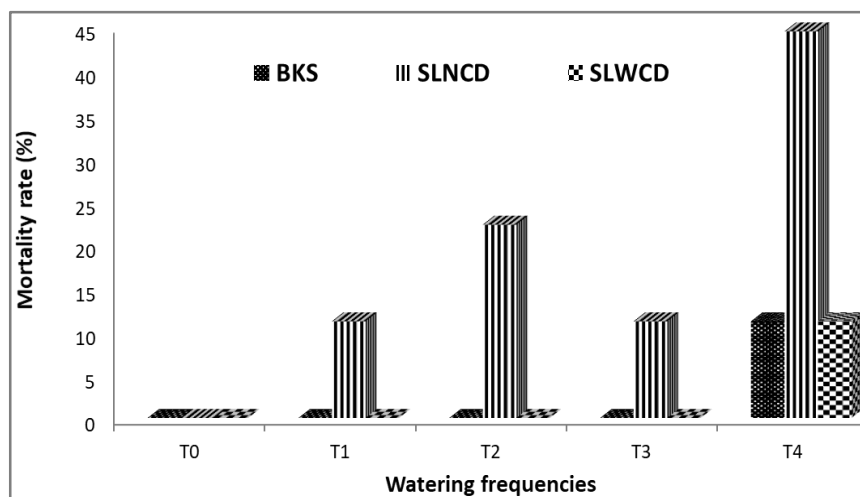


Figure 3. Mortality rates for every watering frequency of the studied substrates. **BKS**, Bokashi; **SLWCD**, Sandy loam with cow dung; **SLNCD**, Sandy loam without cow dung; **T0**, Permanently wet; **T1**, Watering once every 2 days; **T2**, Watering once every 3 days; **T3**, Watering once every 4 days; **T4**, Watering once every 5 days.

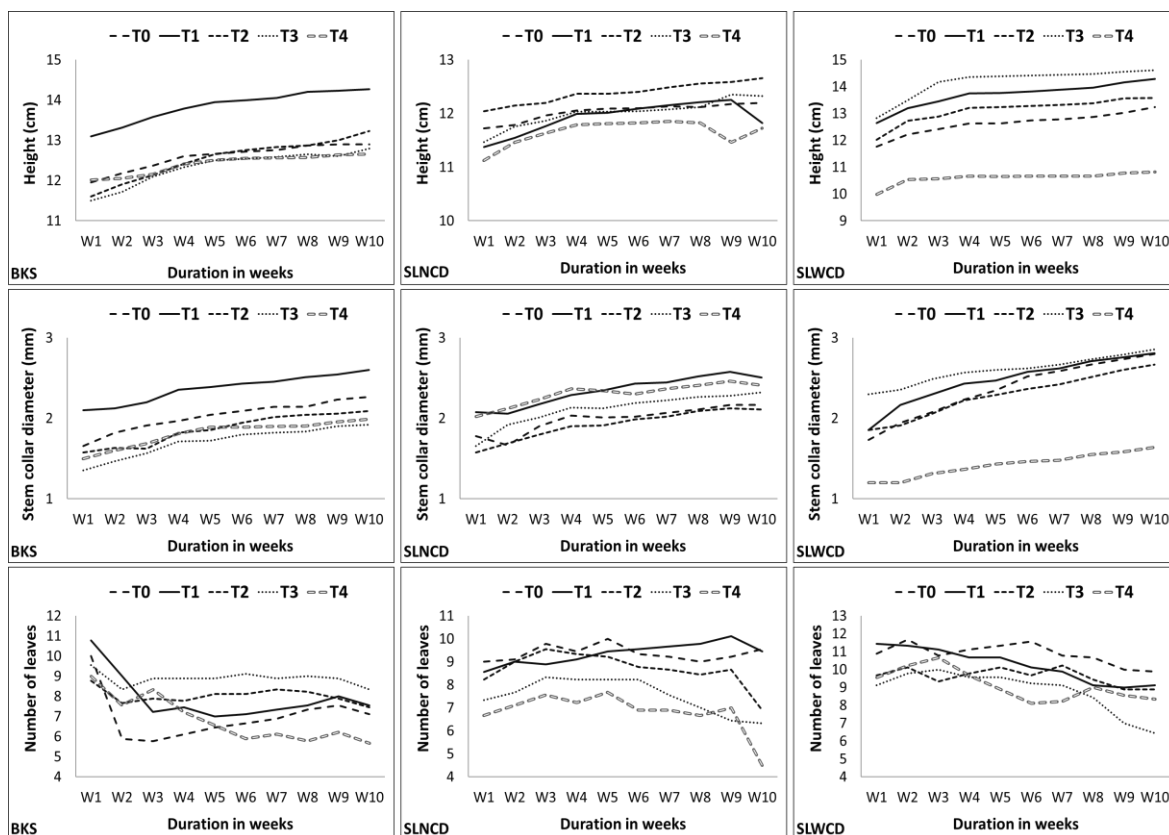


Figure 4. Seedlings' height, stem collar diameter and leaf biomass curves as function of the substrates and watering frequencies. **BKS**, Bokashi; **SLWCD**, Sandy loam with cow dung; **SLNCD**, Sandy loam without cow dung; **T0**, Permanently wet; **T1**, Watering once every 2 days; **T2**, Watering once every 3 days; **T3**, Watering once every 4 days; **T4**, Watering once every 5 days.

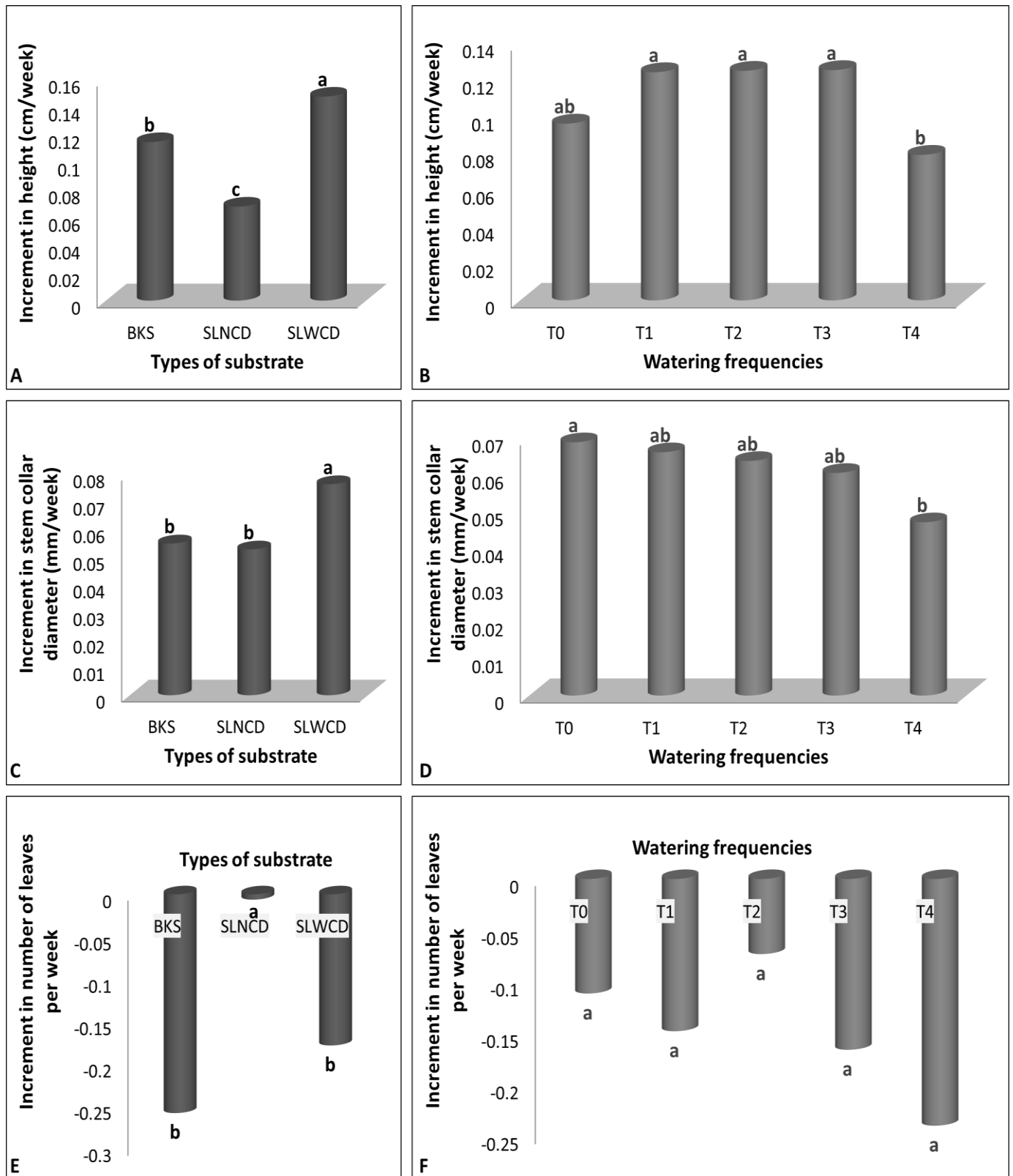


Figure 5. Turkey pairwise comparison tests results on seedlings' growth parameters for studied factors taken separately. **BKS**, Bokashi; **SLWCD**, Sandy loam with cow dung; **SLNCD**, Sandy loam without cow dung; **T0**, Permanently wet; **T1**, Watering once every 2 days; **T2**, Watering once every 3 days; **T3**, Watering once every 4 days; **T4**, Watering once every 5 days.

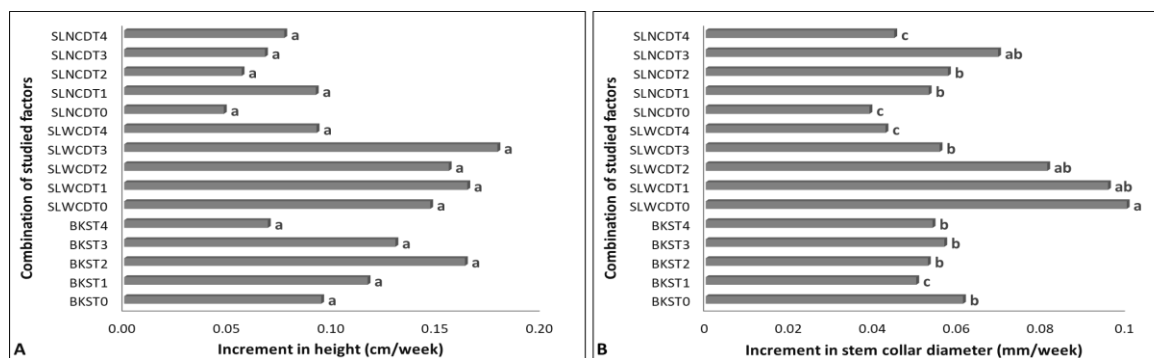


Figure 6. Turkey pairwise comparison tests results on seedlings' growth parameters for the interaction of studied factors. **BKS**, Bokashi; **SLWCD**, Sandy loam with cow dung; **SLNCD**, Sandy loam without cow dung; **T0**, Permanently wet; **T1**, Watering once every 2 days; **T2**, Watering once every 3 days; **T3**, Watering once every 4 days; **T4**, Watering once every 5 days.

in combination with watering frequency T3 (SLWCDT3), while the lowest was on SLNCD. Therefore, it is deduced that sandy loam substrate with cow dung watered once every 2 to 4 days induced a better height growth performance of *T. tetrapleura* seedlings. Regarding the stem collar diameter growth, the results of the analysis revealed significant differences between the effect of the substrates, watering frequencies and the interaction of the two factors (Table 3). The Tukey test allowed us to highlight these differences between the substrates SLNCD-BKS and SLNCD-SLWCD (Figure 5C).

In terms of watering frequencies, the significant difference was found between T4 and T0 (Figure 5D). In terms of interaction between the two factors, it was revealed through the analysis that, the sandy loam substrate with cow dung in combination with the watering frequencies T0, T1 and T2 created better conditions for the growth in stem collar diameter of seedlings (0.11 cm/week, 0.09 cm/week, and 0.08 cm/week respectively). The lowest gain was obtained for seedlings on sandy loam substrate without cow dung combined with watering frequency T0. Therefore, it could be deduced that sandy loam substrate with cow dung watered once every 1 to 3 days provides better performance in seedling stem collar diameter growth (Figure 6).

For the leaf biomass, the analysis showed a significant influence of substrates on the increment in seedling leaves number (Table 3) especially between SLNCD and BKS (Figure 5E). On the substrate SLNCD, the plants kept their leaves better leading to the conclusion that, unfertilized substrate allows *T. Tetrapleura* seedlings in nursery to better keep their leaves.

DISCUSSION

The results of our study showed no mortality of the seedlings for the three substrates regardless of the

watering frequencies during the first seven weeks of the experiment. But, from the 8th week, mortality cases stated being recorded mainly for the watering frequency T4 and later for the other watering frequencies except T0 which provided permanent moisture to substrates for seedlings. Water is extremely important for the survival and development of plants as it allows the transport of nutrients from the soil into the plants and participates in the chemical and biological processes that take place in plants species. Given its importance, when it lacks in substrates plants are living on, their response is mainly reflected in leaves and roots that show signs of etiolation, atrophy, curling, senescence and even abscission (Patharkar and Walker, 2019; Bhusal et al., 2020) leading in some cases to stunted leaf growth. In the event of continuous lack of water, plants usually reduce their leaf biomass by losing some leaves to diminish the areas involved in evapotranspiration process and therefore limit water loss (Nadal et al., 2020; Wu et al., 2022). This explains the depletion of leaf biomass observed during the first weeks of the experiment through the trend of seedlings' leaf biomass curves (Figure 3). Some plant species can withstand absence of water for several days, but others not. This is mainly the case of species growing in arid climate (Vahdati et al., 2017). Plant development stage also influence its capacity to resist and adapt to water deficiency as seedlings and younger plants tend to not withstand longer period of lack of water compared to mature trees due to the large and well-developed rooting system of mature trees that permit deep soil prospection for water supply (Padilla and Pugnaire, 2007). The structure of the soil in addition to the presence of organic matter as well as organic matter quantity and quality can improve soil's capacity to absorb water and make it available for plants over relatively longer period after watering. This justifies the highest mortality that occurred on the sandy loam substrate without cow dung. Indeed, as being unfertilized substrate and mainly composed of sand and silt that do not have greater water

storage capacity compared to clay, a lack of water has easily led to the wilting and death of seedlings as they grew and expressed a growing and unmet need for water (Nadal et al., 2020; Wu et al., 2022). It is also important to point out water evaporation as mean of water loss from soil and therefore, from substrates especially as function of the soil particles sizes in the substrates (Lichner et al., 2020).

The sandy loam substrate with cow dung, followed by the bokashi substrate, exhibited a globally higher performance of seedlings' growth in height and stem collar diameter compared to the sandy loam substrate without cow dung. These two substrates contained fertilizers in the form of manure from manly plants by-products for the bokashi and animal for the sandy loam substrate with cow dung that enriched the soil with nutrients like nitrogen causing faster growth for *T. tetraptera* seedlings. In addition to water availability, to ensure survival of plants, fertile soil is necessary for the speedy growth of seedlings. Organic manure has porous and spongy structure thus helping soil to increase their aeration and contribute to improving soil physical and biological properties as well as enhancing soil fertility (Usman et al., 2019 Kiran et al., 2007; Indriyani et al., 2011; Tchabi et al., 2012; Akintola et al., 2021; Tokorou-Orou-Mere et al., 2021). However, some studies have revealed stronger positive impact of animal manure such as cow dung on seedlings' height and biomass growth in nurseries as well as trees in plantation due to the amount and quality of nutrients it contains (Foidl et al., 2001; Tchabi et al., 2012; Ognalaga et al., 2015).

The results from the combined effect of substrate and water on growth performances showed that the best growth in height is obtained on the sandy loam substrate with cow dung and T1 to T3 against the stronger growth in stem collar diameter obtained with T0. This suggests that *T. tetraptera* height growth is governed by the type of substrate and its stem collar diameter growth by the water supply.

Several studies have shown the positive impact of organic fertilizer on pants growth. However, some plant species especially species of the Legume Family, are known to fix from the air nitrogen which is substantial for plant growth through nitrogen-fixing symbiosis with soil bacteria called rhizobia (Roy et al., 2022). But even though *T. tetraptera* belong to the family of Fabaceae which is a Legume Family, the growth of seedlings on the unfertilized substrate was significantly lower than the growth of seedlings on the fertilized substrates. This points out the fact that *T. tetraptera* seedlings were unable to fix nitrogen from the atmosphere at their stage of development. The seedlings haven't found in the substrate used, the appropriate bacteria like rhizobia to establish this win-win symbiotic relationship with. This could also imply that if inoculation was conducted, the fertilized and unfertilized substrates might have induced similar growth performances on the species seedlings. Inoculation nowadays is used on legume plant species (Arshad et al.,

2008; de Vasconcelos Martins Ferreira et al., 2020) and also extended to non-legume plant species to improve growth and yield in agricultural domain (Xu et al., 2014). The benefits of inoculation go beyond growth improvement as it allows plant species to adapt to drought conditions as pointed out in studies on *Pisum sativum* (Arshad et al., 2008).

Conclusion

From the current study, it could be deduced that, *T. tetraptera* survival and growth in nursery is influenced by the type of substrates as well as the watering frequencies. The early mortality cases occurred from the 8th weeks with the highest mortality rates recorded for the watering frequency of one watering every 5 days. The sandy loam substrate without cow dung led to the lowest survival rate. Regarding seedlings' growth performances, fertilized substrates have proven suitable for improved height and stem diameter increment compared to the unfertilized substrate. Among the fertilized substrates, the animal manure substrate appeared the best. From the statistical analysis, *T. tetraptera* seedlings perform well on fertilized substrates with watering frequencies ranged from one watering every day to one every 3 days. Therefore, these types of substrate and watering frequencies are recommended for growing *T. tetraptera* seedlings in nursery for improved growth performances and sustainable water use.

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